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TITLE: Modulators of .beta.-amyloid peptide aggregation comprising D-amino acids

Abstract Text (1):
Compounds that modulate natural .beta. amyloid peptide aggregation are provided. The modulators of the invention comprise a peptide, preferably based on a .beta. amyloid peptide, that is comprised entirely of D-amino acids. Preferably, the peptide comprises 3-5 D-amino acid residues and includes at least two D-amino acid residues independently selected from the group consisting of D-leucine, D-phenylalanine and D-valine. In a particularly preferred embodiment, the peptide is a retro-inverso isomer of a .beta. amyloid peptide, preferably a retro-inverso isomer of A.beta..sub.17-21. In certain embodiments, the peptide is modified at the amino-terminus, the carboxy-terminus, or both. Preferred amino-terminal modifying groups include cyclic, heterocyclic, polycyclic and branched alkyl groups. Preferred carboxy-terminal modifying groups include an amide group, an alkyl amide group, an aryl amide group or a hydroxy group. Pharmaceutical compositions comprising the compounds of the invention, and diagnostic and treatment methods for amyloidogenic diseases using the compounds of the invention, are also disclosed.

Brief Summary Text (2):
Alzheimer's disease (AD), first described by the Bavarian psychiatrist Alois Alzheimer in 1907, is a progressive neurological disorder that begins with short term memory loss and proceeds to disorientation, impairment of judgment and reasoning and, ultimately, dementia. The course of the disease usually leads to death in a severely debilitated, immobile state between four and 12 years after onset. AD has been estimated to afflict 5 to 11 percent of the population over age 65 and as much as 47 percent of the population over age 85. The societal cost for managing AD is upwards of 80 billion dollars annually, primarily due to the extensive custodial care required for AD patients. Moreover, as adults born during the population boom of the 1940's and 1950's approach the age when AD becomes more prevalent, the control and treatment of AD will become an even more significant health care problem. Currently, there is no treatment that significantly retards the progression of the disease. For reviews on AD, see Selkoe, D. J. Sci. Amer., November 1991, pp. 68-78; and Yankner, B. A. et al. (1991) N. Eng. J. Med. 325:1849-1857.

Brief Summary Text (3):
It has recently been reported (Games et al. (1995) Nature 373:523-527) that an Alzheimer-type neuropathology has been created in transgenic mice. The transgenic mice express high levels of human mutant amyloid precursor protein and progressively develop many of the pathological conditions associated with AD.

Brief Summary Text (4):
Pathologically, AD is characterized by the presence of distinctive lesions in the victim's brain. These brain lesions include abnormal intracellular filaments called neurofibrillary tangles (NTFs) and extracellular deposits of amyloidogenic proteins in senile, or amyloid, plaques. Amyloid deposits are also present in the walls of cerebral blood vessels of AD patients. The major protein constituent of amyloid plaques has been identified as a 4 kilodalton peptide called .beta.-amyloid peptide (.beta.-AP)(Glenner, G. G. and Wong, C. W. (1984) Biochem. Biophys. Res. Commun. 120:885-890; Masters, C. et al. (1985) Proc. Natl. Acad Sci. USA 82:4245-4249). Diffuse deposits of .beta.-AP are frequently observed in normal adult brains, whereas AD brain tissue is characterized by more compacted, dense-core .beta.-amyloid plaques. (See e.g., Davies, L. et al. (1988) Neurology 38:1688-1693). These observations suggest that .beta.-AP deposition precedes, and contributes to, the destruction of neurons that occurs in AD. In further support of a direct pathogenic role for .beta.-AP, .beta.-amyloid has been shown to be toxic to

mature neurons, both in culture and in vivo. Yankner, B. A. et al. (1989) Science 245:417-420; Yankner, B. A. et al. (1990) Proc. Natl. Acad. Sci. USA 87:9020-9023; Roher, A. E. et al. (1991) Biochem. Biophys. Res. Commun. 174:572-579; Kowall, N. W. et al. (1991) Proc. Natl. Acad. Sci. USA 88:7247-7251. Furthermore, patients with hereditary cerebral hemorrhage with amyloidosis-Dutch-type (HCHWA-D), which is characterized by diffuse .beta.-amyloid deposits within the cerebral cortex and cerebrovasculature, have been shown to have a point mutation that leads to an amino acid substitution within .beta.-AP. Levy, E. et al. (1990) Science 248:1124-1126. This observation demonstrates that a specific alteration of the .beta.-AP sequence can cause .beta.-amyloid to be deposited.

Brief Summary Text (5):
Natural .beta.-AP is derived by proteolysis from a much larger protein called the amyloid precursor protein (APP). Kang, J. et al. (1987) Nature 325:733; Goldgaber, D. et al. (1987) Science 235:877; Robakis, N. K. et al. (1987) Proc. Natl. Acad. Sci. USA 84:4190; Tanzi, R. E. et al. (1987) Science 235:880. The APP gene maps to chromosome 21, thereby providing an explanation for the .beta.-amyloid deposition seen at an early age in individuals with Down's syndrome, which is caused by trisomy of chromosome 21. Mann, D. M. et al. (1989) Neuropathol. Appl. Neurobiol. 15:317; Rumble, B. et al. (1989) N. Eng. J. Med. 320:1446. APP contains a single membrane spanning domain, with a long amino terminal region (about two-thirds of the protein) extending into the extracellular environment and a shorter carboxy-terminal region projecting into the cytoplasm. Differential splicing of the APP messenger RNA leads to at least five forms of APP, composed of either 563 amino acids (APP-563), 695 amino acids (APP-695), 714 amino acids (APP-714), 751 amino acids (APP-751) or 770 amino acids (APP-770).

Brief Summary Text (6):
Within APP, naturally-occurring .beta. amyloid peptide begins at an aspartic acid residue at amino acid position 672 of APP-770. Naturally-occurring .beta.-AP derived from proteolysis of APP is 39 to 43 amino acid residues in length, depending on the carboxy-terminal end point, which exhibits heterogeneity. The predominant circulating form of .beta.-AP in the blood and cerebrospinal fluid of both AD patients and normal adults is .beta.1-40 ("short .beta."). Seubert, P. et al. (1992) Nature 359:325; Shoji, M. et al. (1992) Science 258:126. However, .beta.1-42 and .beta.1-43 ("long .beta.") also are forms in .beta.-amyloid plaques. Masters, C. et al. (1985) Proc. Natl. Acad. Sci. USA 82:4245; Miller, D. et al. (1993) Arch. Biochem. Biophys. 301:41; Mori, H. et al. (1992) J. Biol. Chem. 267:17082. Although the precise molecular mechanism leading to .beta.-APP aggregation and deposition is unknown, the process has been likened to that of nucleation-dependent polymerizations, such as protein crystallization, microtubule formation and actin polymerization. See e.g., Jarrett, J. T. and Lansbury, P. T. (1993) Cell 73:1055-1058. In such processes, polymerization of monomer components does not occur until nucleus formation. Thus, these processes are characterized by a lag time before aggregation occurs, followed by rapid polymerization after nucleation. Nucleation can be accelerated by the addition of a "seed" or preformed nucleus, which results in rapid polymerization. The long .beta. forms of .beta.-AP have been shown to act as seeds, thereby accelerating polymerization of both long and short .beta.-AP forms. Jarrett, J. T. et al. (1993) Biochemistry 32:4693.

Brief Summary Text (7):
In one study, in which amino acid substitutions were made in .beta.-AP, two mutant .beta. peptides were reported to interfere with polymerization of non-mutated .beta.-AP when the mutant and non-mutant forms of peptide were mixed. Hilbich, C. et al. (1992) J. Mol. Biol. 228:460-473. Equimolar amounts of the mutant and non-mutant (i.e., natural) .beta. amyloid peptides were used to see this effect and the mutant peptides were reported to be unsuitable for use in vivo. Hilbich, C. et al. (1992), supra.

Brief Summary Text (9):
This invention pertains to compounds, and pharmaceutical compositions thereof, that can bind to natural .beta. amyloid peptides (.beta.-AP), modulate the aggregation of natural .beta.-AP and/or inhibit the neurotoxicity of natural .beta.-APs. The .beta.-amyloid modulator compounds of the invention comprise a peptidic structure, preferably based on .beta.-amyloid peptide, that is composed entirely of D-amino acids. In various embodiments, the peptidic structure of the modulator compound comprises a D-amino acid sequence corresponding to a L-amino acid sequence found within natural .beta.-AP, a D-amino acid sequence which is a retro-inverso isomer of an L-amino acid sequence found within natural .beta.-AP or a D-amino acid sequence that is a scrambled or substituted version of an L-amino acid sequence found within natural .beta.-AP. Preferably, the D-amino acid peptidic structure of the modulator is designed based upon a subregion of natural .beta.-AP at positions 17-21 (A.beta..sub.17-20 and

↳ anticipates ?13-21; 13-22

A.beta..sub.17-21, respectively), which has the amino acid sequences Leu-Val-Phe-Phe-Ala (SEQ ID NO: 3).

Brief Summary Text (10):
A modulator compound of the invention preferably comprises 3-20 D-amino acids, more preferably 3-10 D-amino acids and even more preferably 3-5 D-amino acids. The D-amino acid peptidic structure of the modulator can have free amino- and carboxy-termini. Alternatively, the amino-terminus, the carboxy-terminus or both may be modified. For example, an N-terminal modifying group can be used that enhances the ability of the compound to inhibit A.beta. aggregation. Moreover, the amino- and/or carboxy termini of the peptide can be modified to alter a pharmacokinetic property of the compound (such as stability, bioavailability and the like). Preferred carboxy-terminal modifying groups include amide groups, alkyl or aryl amide groups (e.g., phenethylamide) and hydroxy groups (i.e., reduction products of peptide acids, resulting in peptide alcohols). Still further, a modulator compound can be modified to label the compound with a detectable substance (e.g., a radioactive label).

Brief Summary Text (18):
Yet another aspect of the invention pertains to methods for inhibiting aggregation of natural .beta.-amyloid peptides. These methods comprise contacting the natural .beta.-amyloid peptides with a modulator compound of the invention such that aggregation of the natural .beta.-amyloid peptides is inhibited.

Brief Summary Text (19):
Yet another aspect of the invention pertains to methods for detecting the presence or absence of natural .beta.-amyloid peptides in a biological sample. These methods comprise contacting a biological sample with a compound of the invention, wherein the compound is labeled with a detectable substance, and detecting the compound bound to natural .beta.-amyloid peptides to thereby detect the presence or absence of natural .beta.-amyloid peptides in the biological sample.

Brief Summary Text (20):
Still another aspect of the invention pertains to methods for treating a subject for a disorder associated with .beta.-amyloidosis. These methods comprise administering to the subject a therapeutically effective amount of a modulator compound of the invention such that the subject is treated for a disorder associated with .beta.-amyloidosis. Preferably, the disorder is Alzheimer's disease. Use of the modulators of the invention for therapy or for the manufacture of a medicament for the treatment of a disorder associated with b-amyloidosis is also encompassed by the invention.

Drawing Description Text (2):
FIG. 1 is a bar graph depicting the stability of an L-amino acid-based modulator compound (PPI-368) and two D-amino acid-based modulator compounds (PPI-433 and PPI-457) in cerebrospinal fluid.

Detailed Description Text (2):
This invention pertains to compounds, and pharmaceutical compositions thereof, that can bind to natural .beta.-amyloid peptides, modulate the aggregation of natural .beta.-amyloid peptides (.beta.-AP) and/or inhibit the neurotoxicity of natural .beta.-APs. A compound of the invention that modulates aggregation of natural .beta.-AP, referred to herein interchangeably as a .beta.-amyloid modulator compound, a .beta.-amyloid modulator or simply a modulator, alters the aggregation of natural .beta.-AP when the modulator is contacted with natural .beta.-AP. Thus, a compound of the invention acts to alter the natural aggregation process or rate for .beta.-AP, thereby disrupting this process. Preferably, the compounds inhibit .beta.-AP aggregation. The compounds of the invention are characterized in that they comprise a peptidic structure composed entirely of D-amino acid residues. This peptidic structure is preferably based on .beta.-amyloid peptide and can comprise, for example, a D-amino acid sequence corresponding to a L-amino acid sequence found within natural .beta.-AP, a D-amino acid sequence which is a retro-inverso isomer of an L-amino acid sequence found within natural .beta.-AP or a D-amino acid sequence that is a scrambled or substituted version of an L-amino acid sequence found within natural .beta.-AP. The invention encompasses modulator compounds comprising a D-amino acid peptidic structure having free amino- and carboxy-termini, as well as modulator compounds in which the amino-terminus, the carboxy-terminus, and/or side chain(s) of the peptidic structure are modified.

Detailed Description Text (3):
The .beta.-amyloid modulator compounds of the invention can be selected based upon their ability to bind to natural .beta.-amyloid peptides, modulate the aggregation of

natural .beta.-AP in vitro and/or inhibit the neurotoxicity of natural .beta.-AP fibrils for cultured cells (using assays described herein). Preferred modulator compounds inhibit the aggregation of natural .beta.-AP and/or inhibit the neurotoxicity of natural .beta.-AP. However, modulator compounds selected based on one or both of these properties may have additional properties in vivo that may be beneficial in the treatment of amyloidosis. For example, the modulator compound may interfere with processing of natural .beta.-AP (either by direct or indirect protease inhibition) or by modulation of processes that produce toxic .beta.-AP, or other APP fragments, in vivo. Alternatively, modulator compounds may be selected based on these latter properties, rather than inhibition of A.beta. aggregation in vitro. Moreover, modulator compounds of the invention that are selected based upon their interaction with natural .beta.-AP also may interact with APP or with other APP fragments. Still further, a modulator compound of the invention can be characterized by its ability to bind to .beta.-amyloid fibrils (which can be determined, for example, by radiolabeling the compound, contacting the compound with .beta.-amyloid plaque and imaging the compound bound to the plaque), while not significantly altering the aggregation of the .beta.-amyloid fibrils. Such a compound that binds efficiently to .beta.-amyloid fibrils while not significantly altering the aggregation of the .beta.-amyloid fibrils can be used, for example, to detect .beta.-amyloid fibrils (e.g., for diagnostic purposes, as described further herein). It should be appreciated, however, that the ability of a particular compound to bind to .beta.-amyloid fibrils and/or modulate their aggregation may vary depending upon the concentration of the compound. Accordingly, a compound that, at a low concentration, binds to .beta.-amyloid fibrils without altering their aggregation may nevertheless inhibit aggregation of the fibrils at a higher concentration. All such compounds having the property of binding to .beta.-amyloid fibrils and/or modulating the aggregation of the fibrils are intended to be encompassed by the invention.

Detailed Description Text (4):
As used herein, a "modulator" of .beta.-amyloid aggregation is intended to refer to an agent that, when contacted with natural .beta. amyloid peptides, alters the aggregation of the natural .beta. amyloid peptides. The term "aggregation of .beta. amyloid peptides" refers to a process whereby the peptides associate with each other to form a multimeric, largely insoluble complex. The term "aggregation" further is intended to encompass .beta. amyloid fibril formation and also encompasses .beta.-amyloid plaques.

Detailed Description Text (5):
The terms "natural .beta.-amyloid peptide", "natural .beta.-AP" and "natural A.beta. peptide", used interchangeably herein, are intended to encompass naturally occurring proteolytic cleavage products of the .beta. amyloid precursor protein (APP) which are involved in .beta.-AP aggregation and .beta.-amyloidosis. These natural peptides include .beta.-amyloid peptides having 39-43 amino acids (i.e., A.beta..sub.1-39, A.beta..sub.1-40, A.beta..sub.1-41, A.beta..sub.1-42 and A.beta..sub.1-43). The amino-terminal amino acid residue of natural .beta.-AP corresponds to the aspartic acid residue at position 672 of the 770 amino acid residue form of the amyloid precursor protein ("APP-770"). The 43 amino acid long form of natural .beta.-AP has the amino acid sequence

Detailed Description Text (6):
(also shown in SEQ ID NO: 1), whereas the shorter forms have 1-4 amino acid residues deleted from the carboxy-terminal end. The amino acid sequence of APP-770 from position 672 (i.e., the amino-terminus of natural .beta.-AP) to its C-terminal end (103 amino acids) is shown in SEQ ID NO: 2. The preferred form of natural .beta.-AP for use in the aggregation assays described herein is A.beta..sub.1-40.

Detailed Description Text (7):
In the presence of a modulator of the invention, aggregation of natural .beta. amyloid peptides is "altered" or "modulated". The various forms of the term "alteration" or "modulation" are intended to encompass both inhibition of .beta.-AP aggregation and promotion of .beta.-AP aggregation. Aggregation of natural .beta.-AP is "inhibited" in the presence of the modulator when there is a decrease in the amount and/or rate of .beta.-AP aggregation as compared to the amount and/or rate of .beta.-AP aggregation in the absence of the modulator. The various forms of the term "inhibition" are intended to include both complete and partial inhibition of .beta.-AP aggregation. Inhibition of aggregation can be quantitated as the fold increase in the lag time for aggregation or as the decrease in the overall plateau level of aggregation (i.e., total amount of aggregation), using an aggregation assay as described in the Examples. In various embodiments, a modulator of the invention increases the lag time of aggregation at least 1.2-fold, 1.5-fold, 1.8-fold, 2-fold, 2.5-fold, 3-fold, 4-fold or 5-fold. In

various other embodiments, a modulator of the invention inhibits the plateau level of aggregation at least 10%, 20%, 30%, 40%, 50%, 75% or 100%.

Detailed Description Text (8):
A modulator which inhibits .beta.-AP aggregation (an "inhibitory modulator compound") can be used to prevent or delay the onset of .beta.-amyloid deposition. Preferably, inhibitory modulator compounds of the invention inhibit the formation and/or activity of neurotoxic aggregates of natural A.beta. peptide (i.e., the inhibitory compounds can be used to inhibit the neurotoxicity of .beta.-AP). Additionally, the inhibitory compounds of the invention preferably reduce the neurotoxicity of preformed .beta.-AP aggregates, indicating that the inhibitory modulators can either bind to preformed A.beta. fibrils or soluble aggregate and modulate their inherent neurotoxicity or that the modulators can perturb the equilibrium between monomeric and aggregated forms of .beta.-AP in favor of the non-neurotoxic form.

Detailed Description Text (9):
Alternatively, in another embodiment, a modulator compound of the invention promotes the aggregation of natural A.beta. peptides. The various forms of the term "promotion" refer to an increase in the amount and/or rate of .beta.-AP aggregation in the presence of the modulator, as compared to the amount and/or rate of .beta.-AP aggregation in the absence of the modulator. Such a compound which promotes A.beta. aggregation is referred to as a stimulatory modulator compound. Stimulatory modulator compounds may be useful for sequestering .beta.-amyloid peptides, for example in a biological compartment where aggregation of .beta.-AP may not be deleterious to thereby deplete .beta.-AP from a biological compartment where aggregation of .beta.-AP is deleterious. Moreover, stimulatory modulator compounds can be used to promote A.beta. aggregation in in vitro aggregation assays (e.g., assays such as those described in Example 2), for example in screening assays for test compounds that can then inhibit or reverse this A.beta. aggregation (i.e., a stimulatory modulator compound can act as a "seed" to promote the formation of A.beta. aggregates).

Detailed Description Text (10):
In a preferred embodiment, the modulators of the invention are capable of altering .beta.-AP aggregation when contacted with a molar excess amount of natural .beta.-AP. A "molar excess amount of natural .beta.-AP" refers to a concentration of natural .beta.-AP, in moles, that is greater than the concentration, in moles, of the modulator. For example, if the modulator and .beta.-AP are both present at a concentration of 1 .mu.M, they are said to be "equimolar", whereas if the modulator is present at a concentration of 1 .mu.M and the .beta.-AP is present at a concentration of 5 .mu.M, the .beta.-AP is said to be present at a 5-fold molar excess amount compared to the modulator. In preferred embodiments, a modulator of the invention is effective at altering natural .beta.-AP aggregation when the natural .beta.-AP is present at at least a 2-fold, 3-fold or 5-fold molar excess compared to the concentration of the modulator. In other embodiments, the modulator is effective at altering .beta.-AP aggregation when the natural .beta.-AP is present at at least a 10-fold, 20-fold, 33-fold, 50-fold, 100-fold, 500-fold or 1000-fold molar excess compared to the concentration of the modulator.

Detailed Description Text (11):
As used herein, the term ".beta. amyloid peptide comprised entirely of D-amino acids", as used in a modulator of the invention, is intended to encompass peptides having an amino acid sequence identical to that of the natural sequence in APP, as well as peptides having acceptable amino acid substitutions from the natural sequence, but which is composed of D-amino acids rather than the natural L-amino acids present in natural .beta.-AP. Acceptable amino acid substitutions are those that do not affect the ability of the D-amino acid-containing peptide to alter natural .beta.-AP aggregation. Moreover, particular amino acid substitutions may further contribute to the ability of the peptide to alter natural .beta.-AP aggregation and/or may confer additional beneficial properties on the peptide (e.g., increased solubility, reduced association with other amyloid proteins, etc.). A peptide having an identical amino acid sequence to that found within a parent peptide but in which all L-amino acids have been substituted with all D-amino acids is also referred to as an "inverso" compounds. For example, if a parent peptide is Thr-Ala-Tyr, the inverso form is D-Thr-D-Ala-D-Tyr.

Detailed Description Text (12):
As used herein, the term "retro-inverso isomer of a .beta. amyloid peptide", as used in a modulator of the invention, is intended to encompass peptides in which the sequence of the amino acids is reversed as compared to the sequence in natural .beta.-AP and all L-amino acids are replaced with D-amino acids. For example, if a parent peptide is

Thr-Ala-Tyr, the retro-inverso form is D-Tyr-D-Ala-D-Thr. Compared to the parent peptide, a retro-inverso peptide has a reversed backbone while retaining substantially the original spatial conformation of the side chains, resulting in a retro-inverso isomer with a topology that closely resembles the parent peptide. See Goodman et al. "Perspectives in Peptide Chemistry" pp. 283-294 (1981). See also U.S. Pat. No. 4,522,752 by Sisto for further description of "retro-inverso" peptides.

Detailed Description Text (15):
In one embodiment, a modulator compound of the invention comprises a .beta.-amyloid peptide, the .beta.-amyloid peptide being comprised entirely of D-amino acids, wherein the compound binds to natural .beta.-amyloid peptides or modulates the aggregation or inhibits the neurotoxicity of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. Preferably, the .beta.-amyloid peptide of the modulator is comprised of 3-20 D-amino acids, more preferably 3-10 D-amino acids, and even more preferably 3-5 D-amino acids. In one embodiment, the .beta.-amyloid peptide of the modulator is amino-terminally modified, for example with a modifying group comprising a cyclic, heterocyclic, polycyclic or branched alkyl group. Examples of suitable N-terminal modifying groups are described further in subsection II below. In another embodiment, the .beta.-amyloid peptide of the modulator is carboxy-terminally modified, for example the modulator can comprise a peptide amide, a peptide alkyl or aryl amide (e.g., a peptide phenethylamide) or a peptide alcohol. Examples of suitable C-terminal modifying groups are described further in subsections II and III below. The .beta.-amyloid peptide of the modulator may be modified to enhance the ability of the modulator to alter .beta.-AP aggregation or neurotoxicity. Additionally or alternatively, .beta.-amyloid peptide of the modulator may be modified to alter a pharmacokinetic property of the modulator and/or to label the modulator with a detectable substance (described further in subsection III below).

Detailed Description Text (16):
In another embodiment, a modulator compound of the invention comprises a retro-inverso isomer of a .beta.-amyloid peptide, wherein the compound binds to natural .beta.-amyloid peptides or modulates the aggregation or inhibits the neurotoxicity of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. Preferably, the retro-inverso isomer of the .beta.-amyloid peptide is comprised of 3-20 D-amino acids, more preferably 3-10 D-amino acids, and even more preferably 3-5 D-amino acids. In one embodiment, the retro-inverso isomer is amino-terminally modified, for example with a modifying group comprising a cyclic, heterocyclic, polycyclic or branched alkyl group. Examples of suitable N-terminal modifying groups are described further in subsection II below. In another embodiment, the retro-inverso isomer is carboxy-terminally modified, for example with an amide group, an alkyl or aryl amide group (e.g., phenethylamide) or a hydroxy group (i.e., the reduction product of a peptide acid, resulting in a peptide alcohol). Examples of suitable C-terminal modifying groups are described further in subsections II and III below. The retro-inverso isomer may be modified to enhance the ability of the modulator to alter .beta.-AP aggregation or neurotoxicity. Additionally or alternatively, the retro-inverso isomer may be modified to alter a pharmacokinetic property of the modulator and/or to label the modulator with a detectable substance (described further in subsection III below).

Detailed Description Text (17):
The modulators of the invention preferably are designed based upon the amino acid sequence of a subregion of natural .beta.-AP. The term "subregion of a natural .beta.-amyloid peptide" is intended to include amino-terminal and/or carboxy-terminal deletions of natural .beta.-AP. The term "subregion of natural .beta.-AP" is not intended to include full-length natural .beta.-AP (i.e., "subregion" does not include A.beta..sub.1-39, A.beta..sub.1-40, A.beta..sub.1-41, A.beta..sub.1-42 and A.beta..sub.1-43). A preferred subregion of natural .beta.-amyloid peptide is an "A.beta. aggregation core domain" (ACD). As used herein, the term "A.beta. aggregation core domain" refers to a subregion of a natural .beta.-amyloid peptide that is sufficient to modulate aggregation of natural .beta.-APs when this subregion, in its L-amino acid form, is appropriately modified (e.g., modified at the amino-terminus), as described in detail in U.S. patent application Ser. No. 08/548,998 and U.S. patent application Ser. No. 08/616,081, the entire contents of each of which are expressly incorporated herein by reference. Preferably, the ACD is modeled after a subregion of natural .beta.-AP that is less than 15 amino acids in length and more preferably is between 3-10 amino acids in length. In various embodiments, the ACD is modeled after a subregion of .beta.-AP that is 10, 9, 8, 7, 6, 5, 4 or 3 amino acids in length. In one embodiment, the subregion of .beta.-AP upon which the ACD is modeled is an internal or carboxy-terminal region of .beta.-AP (i.e., downstream of the amino-terminus at amino

acid position 1). In another embodiment, the ACD is modeled after a subregion of .beta.-AP that is hydrophobic. Preferred A.beta. aggregation core domains encompass amino acid residues 17-20 or 17-21 of natural .beta.-AP (A.beta..sub.17-20 and A.beta..sub.17-21, respectively). The amino acid sequences of A.beta..sub.17-20 and A.beta..sub.17-21 are Leu-Val-Phe-Phe (SEQ ID NO: 8) and Leu-Val-Phe-Phe-Ala (SEQ ID NO: 3), respectively.

Detailed Description Text (18):
As demonstrated in the Examples, D-amino acid-containing modulators designed based upon the amino acid sequences of A.beta..sub.17-20 and A.beta..sub.17-21 are particularly effective inhibitors of A.beta. aggregation. These modulators can comprises a D-amino acid sequence corresponding to the L-amino acid sequence of A.beta..sub.17-20 or A.beta..sub.17-21, a D-amino acid sequence which is a retro-inverso isomer of the L-amino acid sequence of A.beta..sub.17-20 or A.beta..sub.17-21, or a D-amino acid sequence that is a scrambled or substituted version of the L-amino acid sequence of A.beta..sub.17-20 or A.beta..sub.17-21. The D-amino acid-based modulators may have unmodified amino- and/or carboxy-termini or, alternatively, the amino-terminus, the carboxy-terminus, or both, may be modified (described further below). The peptidic structures of effective modulators generally are hydrophobic and are characterized by the presence of at least two D-amino acid structures independently selected from the group consisting of a D-leucine structure, a D-phenylalanine structure and a D-valine structure. An used herein, the term a "D-amino acid structure" (such as a "D-leucine structure", a "D-phenylalanine structure" or a "D-valine structure") is intended to include the D-amino acid, as well as analogues, derivatives and mimetics of the D-amino acid that maintain the functional activity of the compound (discussed further below). For example, the term "D-phenylalanine structure" is intended to include D-phenylalanine as well as D-pyridylalanine and D-homophenylalanine. The term "D-leucine structure" is intended to include D-leucine, as well as substitution with D-valine or other natural or non-natural amino acid having an aliphatic side chain, such as D-norleucine. The term "D-valine structure" is intended to include D-valine, as well as substitution with D-leucine or other natural or non-natural amino acid having an aliphatic side chain.

Detailed Description Text (19):
In other embodiments, the peptidic structure of the modulator comprises at least two D-amino acid structures independently selected from the group consisting of a D-leucine structure, a D-phenylalanine structure, a D-valine structure, a D-alanine structure, a D-tyrosine structure and a D-iodotyrosine structure. In another embodiment, the peptidic structure is comprised of at least three D-amino acid structures independently selected from the group consisting of a D-leucine structure, a D-phenylalanine structure and a D-valine structure. In yet another embodiment, the peptidic structure is comprised of at least three D-amino acid structures independently selected from the group consisting of a D-leucine structure, a D-phenylalanine structure, a D-valine structure, a D-alanine structure, a D-tyrosine structure and a D-iodotyrosine structure. In yet another embodiment, the peptidic structure comprises at least four D-amino acid structures independently selected from the group consisting of a D-leucine structure, a D-phenylalanine structure and a D-valine structure. In yet another embodiment, the peptidic structure is comprised of at least four D-amino acid structures independently selected from the group consisting of a D-leucine structure, a D-phenylalanine structure and a D-valine structure. In a preferred embodiment, the peptidic structure includes a D-amino acid dipeptide selected from the group consisting of D-Phe-D-Phe, D-Phe-D-Tyr, D-Tyr-D-Phe, D-Phe-D-IodoTyr and D-IodoTyr-D-Phe.

Detailed Description Text (20):
In one embodiment, the invention provides a .beta.-amyloid modulator compound comprising a formula (I): ##STR1## wherein Xaa.sub.1, Xaa.sub.2, Xaa.sub.3 and Xaa.sub.4 are each D-amino acid structures and at least two of Xaa.sub.1, Xaa.sub.2, Xaa.sub.3 and Xaa.sub.4 are, independently, selected from the group consisting of a D-leucine structure, a D-phenylalanine structure and a D-valine structure;

Detailed Description Text (21):
Y, which may or may not be present, is a structure having the formula (Xaa).sub.a, wherein Xaa is any D-amino acid structure and a is an integer from 1 to 15;

Detailed Description Text (22):
Z, which may or may not be present, is a structure having the formula (Xaa).sub.b, wherein Xaa is any D-amino acid structure and b is an integer from 1 to 15;

Detailed Description Text (25):

wherein Xaa.sub.1, Xaa.sub.2, Xaa.sub.3, Xaa.sub.4, Y, Z, A and n are selected such that the compound binds to natural .beta.-amyloid peptides or modulates the aggregation or inhibits the neurotoxicity of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides.

Detailed Description Text (26):
In a subembodiment of this formula, a fifth amino acid residue, Xaa.sub.5, is specified C-terminal to Xaa.sub.4 and Z, which may or may not be present, is a structure having the formula (Xaa).sub.b, wherein Xaa is any D-amino acid structure and b is an integer from 1 to 14. Accordingly, the invention provides a .beta.-amyloid modulator compound comprising a formula (II): ##STR2## wherein b is an integer from 1 to 14.

Detailed Description Text (31):
In the modulators of the invention having the formula (I) or (II) shown above, an optional modifying group ("A") is attached directly or indirectly to the peptidic structure of the modulator. (As used herein, the term "modulating group" and "modifying group" are used interchangeably to describe a chemical group directly or indirectly attached to a peptidic structure). For example, a modifying group(s) can be directly attached by covalent coupling to the peptidic structure or a modifying group(s) can be attached indirectly by a stable non-covalent association. In one embodiment of the invention, a modifying group is attached to the amino-terminus of the peptidic structure of the modulator. Alternatively, in another embodiment of the invention, a modifying group is attached to the carboxy-terminus of the peptidic structure of the modulator. In yet another embodiment, a modulating group(s) is attached to the side chain of at least one amino acid residues of the peptidic structure of the modulator (e.g., through the epsilon amino group of a lysyl residue(s), through the carboxyl group of an aspartic acid residue(s) or a glutamic acid residue(s), through a hydroxy group of a tyrosyl residue(s), a serine residue(s) or a threonine residue(s) or other suitable reactive group on an amino acid side chain).

Detailed Description Text (32):
If a modifying group(s) is present, the modifying group is selected such that the compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. Accordingly, since the .beta.-AP peptide of the compound is modified from its natural state, the modifying group "A" as used herein is not intended to include hydrogen. In a modulator of the invention, a single modifying group may be attached to the peptidic structure or multiple modifying groups may be attached to the peptidic structure. The number of modifying groups is selected such that the compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. However, n preferably is an integer between 1 and 60, more preferably between 1 and 30 and even more preferably between 1 and 10 or 1 and 5. In a preferred embodiment, A is an amino-terminal modifying group comprising a cyclic, heterocyclic, polycyclic or branched alkyl group and n=1. In another preferred embodiment, A is carboxy-terminally modifying group comprising an amide group, an alkyl amide group, an aryl amide group or a hydroxy group, and n=1. Suitable modifying groups are described further in subsections II and III below.

Detailed Description Text (33):
In another embodiment, the invention provides a .beta.-amyloid modulator compound comprising a formula (III):

Detailed Description Text (34):
wherein Xaa.sub.1, Xaa.sub.2, Xaa.sub.3 and Xaa.sub.4 are each D-amino acid structures and at least two of Xaa.sub.1, Xaa.sub.2, Xaa.sub.3 and Xaa.sub.4 are, independently, selected from the group consisting of a D-leucine structure, a D-phenylalanine structure and a D-valine structure;

Detailed Description Text (35):
Y, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.a, wherein Xaa is any amino acid structure and a is an integer from 1 to 15;

Detailed Description Text (36):
Z, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.b, wherein Xaa is any amino acid structure and b is an integer from 1 to 15; and

Detailed Description Text (39):
Xaa.sub.1, Xaa.sub.2, Xaa.sub.3, Xaa.sub.4, Y, Z, A and B being selected such that the compound binds to natural .beta.-amyloid peptides or modulates the aggregation or

inhibits the neurotoxicity of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides.

Detailed Description Text (40):
In a subembodiment of formula (III), a fifth amino acid residue, Xaa.sub.5, is specified C-terminal to Xaa.sub.4 and Z, which may or may not be present, is a structure having the formula (Xaa).sub.b, wherein Xaa is any D-amino acid structure and b is an integer from 1 to 14. Accordingly, the invention provides a .beta.-amyloid modulator compound comprising a formula (IV):

Detailed Description Text (47):
In preferred specific embodiments, the invention provides a .beta.-amyloid modulator compound comprising a peptidic structure selected from the group consisting of D-Leu-D-Val-D-Phe-D-Phe (SEQ ID NO: 9), D-Leu-D-Val-D-Phe-phenethylamide (SEQ ID NO: 10), D-Leu-D-Val-D-Tyr-D-Phe (SEQ ID NO: 11), D-Leu-D-Val-D-IodoTyr-D-Phe (SEQ ID NO: 12), D-Leu-D-Val-D-Phe-D-Tyr (SEQ ID NO: 13), D-Leu-D-Val-D-Phe-D-IodoTyr (SEQ ID NO: 14), D-Leu-D-Val-D-Phe-D-Ala (SEQ ID NO: 15), D-Leu-D-Val-D-Phe-D-Phe-D-Ala (SEQ ID NO: 16), D-Ala-D-Val-D-Phe-D-Phe-D-Leu (SEQ ID NO: 17), D-Leu-D-Val-D-Tyr-D-Phe-D-Ala (SEQ ID NO: 18), D-Leu-D-Val-D-IodoTyr-D-Phe-D-Ala (SEQ ID NO: 19), D-Leu-D-Val-D-Phe-D-Tyr-D-Ala (SEQ ID NO: 20), D-Leu-D-Val-D-Phe-D-IodoTyr-D-Ala (SEQ ID NO: 21), D-Phe-D-Phe-D-Val-D-Leu (SEQ ID NO: 22), D-Ala-D-Phe-D-Phe-D-Val (SEQ ID NO: 23), D-Ala-D-Phe-D-Phe-D-Val-D-Leu (SEQ ID NO: 4), D-Ala-D-Phe-D-Phe-D-Leu-D-Leu (SEQ ID NO: 5), D-Leu-D-Phe-D-Phe-D-Val-D-Leu (SEQ ID NO: 6), D-Phe-D-Phe-D-Phe-D-Val-D-Leu (SEQ ID NO: 7), D-Phe-D-Phe-D-Phe-D-Leu-D-Val (SEQ ID NO: 24), D-Phe-D-Phe-D-Phe-D-Phe-D-Leu (SEQ ID NO: 25) and D-Ala-D-Phe-D-Phe-D-Phe-D-Leu (SEQ ID NO: 26). Any of the aforementioned specific peptidic structures can be amino-terminally and/or carboxy-terminally modified and described further in subsections II and/or III below.

Detailed Description Text (48):
Particularly preferred modulators comprise D-amino acid peptide amides designed based on the retro-inverso isomer of A.beta..sub.17-21, or acceptable substitutions thereof, including compounds selected from the group consisting of
D-Ala-D-Phe-D-Phe-D-Val-D-Leu-amide (SEQ ID NO: 4; C-terminal amide),
D-Ala-D-Phe-D-Phe-D-Leu-D-Leu-amide (SEQ ID NO: 5; C-terminal amide),
D-Leu-D-Phe-D-Phe-D-Val-D-Leu-amide (SEQ ID NO: 6; C-terminal amide) and
D-Phe-D-Phe-D-Phe-D-Val-D-Leu-amide (SEQ ID NO: 7; C-terminal amide),
D-Phe-D-Phe-D-Phe-D-Leu-D-Val-amide (SEQ ID NO: 24; C-terminal amide),
D-Phe-D-Phe-D-Phe-D-Phe-D-Leu-amide (SEQ ID NO: 25; C-terminal amide) and
D-Ala-D-Phe-D-Phe-D-Phe-D-Leu-amide (SEQ ID NO: 26; C-terminal amide).

Detailed Description Text (49):
The D-amino acid peptidic structures of the modulators of the invention are further intended to include other peptide modifications, including analogues, derivatives and mimetics, that retain the ability of the modulator to alter natural .beta.-AP aggregation as described herein. For example, a D-amino acid peptidic structure of a modulator of the invention may be further modified to increase its stability, bioavailability, solubility, etc. The terms "analogue", "derivative" and "mimetic" as used herein are intended to include molecules which mimic the chemical structure of a D-peptidic structure and retain the functional properties of the D-peptidic structure. Approaches to designing peptide analogs, derivatives and mimetics are known in the art. For example, see Farmer, P. S. in Drug Design (E. J. Ariens, ed.) Academic Press, New York, 1980, vol. 10, pp. 119-143; Ball. J. B. and Alewood, P. F. (1990) J. Mol. Recognition 3:55; Morgan, B. A. and Gainor, J. A. (1989) Ann. Rep. Med. Chem. 24:243; and Freidinger, R. M. (1989) Trends Pharmacol. Sci. 10:270. See also Sawyer, T. K. (1995) "Peptidomimetic Design and Chemical Approaches to Peptide Metabolism" in Taylor, M. D. and Amidon, G. L. (eds.) Peptide-Based Drug Design: Controlling Transport and Metabolism, Chapter 17; Smith, A. B. 3rd, et al. (1995) J. Am. Chem. Soc. 117:11113-11123; Smith, A. B. 3rd, et al. (1994) J. Am. Chem. Soc. 116:9947-9962; and Hirschman, R., et al. (1993) J. Am. Chem. Soc. 115:12550-12568.

Detailed Description Text (50):
As used herein, a "derivative" of a compound X (e.g., a peptide or amino acid) refers to a form of X in which one or more reaction groups on the compound have been derivatized with a substituent group. Examples of peptide derivatives include peptides in which an amino acid side chain, the peptide backbone, or the amino- or carboxy-terminus has been derivatized (e.g., peptidic compounds with methylated amide linkages). As used herein an "analogue" of a compound X refers to a compound which retains chemical structures of X necessary for functional activity of X yet which also

contains certain chemical structures which differ from X. An examples of an analogue of a naturally-occurring peptide is a peptide which includes one or more non-naturally-occurring amino acids. As used herein, a "mimetic" of a compound X refers to a compound in which chemical structures of X necessary for functional activity of X have been replaced with other chemical structures which mimic the conformation of X. Examples of peptidomimetics include peptidic compounds in which the peptide backbone is substituted with one or more benzodiazepine molecules (see e.g., James, G. L. et al. (1993) Science 260:1937-1942).

Detailed Description Text (51):
Analogues of the modulator compounds of the invention are intended to include compounds in which one or more D-amino acids of the peptidic structure are substituted with a homologous amino acid such that the properties of the original modulator are maintained. Preferably conservative amino acid substitutions are made at one or more amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), .beta.-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Non-limiting examples of homologous substitutions that can be made in the peptidic structures of the modulators of the invention include substitution of D-phenylalanine with D-tyrosine, D-pyridylalanine or D-homophenylalanine, substitution of D-leucine with D-valine or other natural or non-natural amino acid having an aliphatic side chain and/or substitution of D-valine with D-leucine or other natural or non-natural amino acid having an aliphatic side chain.

Detailed Description Text (54):
The modulator compounds of the invention can be incorporated into pharmaceutical compositions (described further in subsection V below) and can be used in detection and treatment methods as described further in subsection VI below.

Detailed Description Text (56):
In certain embodiments of the modulator compounds of the invention, a D-amino acid peptidic structure (such as an A.beta. derived peptide, or an A.beta. aggregation core domain, or an amino acid sequence corresponding to a rearranged A.beta. aggregation core domain) is coupled directly or indirectly to at least one modifying group (abbreviated as MG). The term "modifying group" is intended to include structures that are directly attached to the D-amino acid peptidic structure (e.g., by covalent coupling), as well as those that are indirectly attached to the peptidic structure (e.g., by a stable non-covalent association or by covalent coupling to additional amino acid residues, or mimetics, analogues or derivatives thereof, which may flank the A.beta.-derived D-amino acid peptidic structure). For example, the modifying group can be coupled to the amino-terminus or carboxy-terminus of an A.beta.-derived D-amino acid peptidic structure, or to a peptidic or peptidomimetic region flanking the core domain. Alternatively, the modifying group can be coupled to a side chain of at least one D-amino acid residue of an A.beta.-derived D-amino acid peptidic structure, or to a peptidic or peptidomimetic region flanking the core domain (e.g., through the epsilon amino group of a lysyl residue(s), through the carboxyl group of an aspartic acid residue(s) or a glutamic acid residue(s), through a hydroxy group of a tyrosyl residue(s), a serine residue(s) or a threonine residue(s) or other suitable reactive group on an amino acid side chain). Modifying groups covalently coupled to the D-amino acid peptidic structure can be attached by means and using methods well known in the art for linking chemical structures, including, for example, amide, alkylamino, carbamate, urea or ester bonds.

Detailed Description Text (57):
The term "modifying group" is intended to include groups that are not naturally coupled to natural A.beta. peptides in their native form. Accordingly, the term "modifying group" is not intended to include hydrogen. The modifying group(s) is selected such that the modulator compound alters, and preferably inhibits, aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides or inhibits the neurotoxicity of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. Although not intending to be limited by mechanism, in embodiments where the modulator comprises a modifying group(s), the modifying group(s) is thought to function as a key pharmacophore that enhances the ability of the

modulator to disrupt A.beta. polymerization.

Detailed Description Text (61):
A preferred polycyclic group is a group containing a cis-decalin structure. Although not intending to be limited by mechanism, it is thought that the "bent" conformation conferred on a modifying group by the presence of a cis-decalin structure contributes to the efficacy of the modifying group in disrupting A.beta. polymerization. Accordingly, other structures which mimic the "bent" configuration of the cis-decalin structure can also be used as modifying groups. An example of a cis-decalin containing structure that can be used as a modifying group is a cholanoyl structure, such as a cholyl group. For example, a modulator compound can be modified at its amino terminus with a cholyl group by reacting the aggregation core domain with cholic acid, a bile acid. Moreover, a modulator compound can be modified at its carboxy terminus with a cholyl group according to methods known in the art (see e.g., Wess, G. et al. (1993) Tetrahedron Letters, 34:817-822; Wess, G. et al. (1992) Tetrahedron Letters 33:195-198; and Kramer, W. et al. (1992) J. Biol. Chem. 267:18598-18604). Cholyl derivatives and analogues can also be used as modifying groups. For example, a preferred cholyl derivative is Aic (3-(O-aminoethyl-iso)-cholyl), which has a free amino group that can be used to further modify the modulator compound (e.g., a chelation group for .sup.99m Tc can be introduced through the free amino group of Aic). As used herein, the term "cholanoyl structure" is intended to include the cholyl group and derivatives and analogues thereof, in particular those which retain a four-ring cis-decalin configuration. Examples of cholanoyl structures include groups derived from other bile acids, such as deoxycholic acid, lithocholic acid, ursodeoxycholic acid, chenodeoxycholic acid and hyodeoxycholic acid, as well as other related structures such as cholanic acid, bufalin and resibufogenin (although the latter two compounds are not preferred for use as a modifying group). Another example of a cis-decalin containing compound is 5.beta.-cholestan-3.alpha.-ol (the cis-decalin isomer of (+)-dihydrocholesterol). For further description of bile acid and steroid structure and nomenclature, see Nes, W. R. and McKean, M. L. Biochemistry of Steroids and Other Isopentanoids, University Park Press, Baltimore, Md., Chapter 2.

Detailed Description Text (62):
In addition to cis-decalin containing groups, other polycyclic groups may be used as modifying groups. For example, modifying groups derived from steroids or .beta.-lactams may be suitable modifying groups. In one embodiment, the modifying group is a "biotinyl structure", which includes biotinyl groups and analogues and derivatives thereof (such as a 2-iminobiotinyl group). In another embodiment, the modifying group can comprise a "fluorescein-containing group", such as a group derived from reacting an A.beta.-derived peptidic structure with 5-(and 6-)-carboxyfluorescein, succinimidyl ester or fluorescein isothiocyanate. In various other embodiments, the modifying group(s) can comprise an N-acetylneuraminyl group, a trans-4-cotininecarboxyl group, a 2-imino-1-imidazolidineacetyl group, an (S)-(-)-indoline-2-carboxyl group, a (-)-menthoxyacetyl group, a 2-norbornaneacetyl group, a .gamma.-oxo-5-acenaphthenebutyryl, a (-)-2-oxo-4-thiazolidinecarboxyl group, a tetrahydro-3-furoyl group, a 2-iminobiotinyl group, a diethylenetriaminepentaacetyl group, a 4-morpholinecarbonyl group, a 2-thiopheneacetyl group or a 2-thiophenesulfonyl group.

Detailed Description Text (64):
Yet another type of modifying group is a compound that contains a non-natural amino acid that acts as a beta-turn mimetic, such as a dibenzofuran-based amino acid described in Tsang, K. Y. et al. (1994) J. Am. Chem. Soc. 116:3988-4005; Diaz, H and Kelly, J. W. (1991) Tetrahedron Letters 41:5725-5728; and Diaz. H et al. (1992) J. Am. Chem. Soc. 114:8316-8318. An example of such a modifying group is a peptide-aminoethyldibenzofuranyl-proprionic acid (Adp) group (e.g., DDIIL-Adp) (SEQ ID NO: 31). This type of modifying group further can comprise one or more N-methyl peptide bonds to introduce additional steric hindrance to the aggregation of natural .beta.-AP when compounds of this type interact with natural .beta.-AP.

Detailed Description Text (68):
A .beta.-amyloid modulator compound of the invention can be further modified to alter the specific properties of the compound while retaining the ability of the compound to alter A.beta. aggregation and inhibit A.beta. neurotoxicity. For example, in one embodiment, the compound is further modified to alter a pharmacokinetic property of the compound, such as in vivo stability or half-life. In another embodiment, the compound is further modified to label the compound with a detectable substance. In yet another embodiment, the compound is further modified to couple the compound to an additional therapeutic moiety. Schematically, a modulator of the invention comprising a D-amino

acid A.beta. aggregation core domain coupled directly or indirectly to at least one modifying group can be illustrated as MG-ACD, whereas this compound which has been further modified to alter the properties of the modulator can be illustrated as MG-ACD-CM, wherein CM represents an additional chemical modification.

Detailed Description Text (69):
To further chemically modify the compound, such as to alter the pharmacokinetic properties of the compound, reactive groups can be derivatized. For example, when the modifying group is attached to the amino-terminal end of the aggregation core domain, the carboxy-terminal end of the compound can be further modified. Preferred C-terminal modifications include those which reduce the ability of the compound to act as a substrate for carboxypeptidases. Examples of preferred C-terminal modifiers include an amide group (i.e., a peptide amide), an alkyl or aryl amide group (e.g., an ethylamide group or a phenethylamide group) a hydroxy group (i.e., a peptide alcohol) and various non-natural amino acids, such as D-amino acids and .beta.-alanine. Alternatively, when the modifying group is attached to the carboxy-terminal end of the aggregation core domain, the amino-terminal end of the compound can be further modified, for example, to reduce the ability of the compound to act as a substrate for aminopeptidases.

Detailed Description Text (70):
A modulator compound can be further modified to label the compound by reacting the compound with a detectable substance. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, .beta.-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include .sup.14 C, .sup.123 I, .sup.124 I, .sup.125 I, .sup.131 I, .sup.99m Tc, .sup.35 S or .sup.3 H. In a preferred embodiment, a modulator compound is radioactively labeled with .sup.14 C, either by incorporation of .sup.14 C into the modifying group or one or more amino acid structures in the modulator compound. Labeled modulator compounds can be used to assess the in vivo pharmacokinetics of the compounds, as well as to detect A.beta. aggregation, for example for diagnostic purposes. A.beta. aggregation can be detected using a labeled modulator compound either in vivo or in an in vitro sample derived from a subject.

Detailed Description Text (71):
Preferably, for use as an in vivo diagnostic agent, a modulator compound of the invention is labeled with radioactive technetium or iodine. Accordingly, in one embodiment, the invention provides a modulator compound labeled with technetium, preferably .sup.99m Tc. Methods for labeling peptide compounds with technetium are known in the art (see e.g., U.S. Pat. Nos. 5,443,815, 5,225,180 and 5,405,597, all by Dean et al.; Stepniak-Biniakiewicz, D., et al. (1992) J. Med. Chem. 35:274-279; Fritzberg, A. R., et al. (1988) Proc. Natl. Acad. Sci. USA 85:4025-4029; Baidoo, K. E., et al. (1990) Cancer Res. Suppl. 50:799s-803s; and Regan, L. and Smith, C. K. (1995) Science 270:980-982). A modifying group can be chosen that provides a site at which a chelation group for .sup.99m Tc can be introduced, such as the Aic derivative of cholic acid, which has a free amino group. In another embodiment, the invention provides a modulator compound labeled with radioactive iodine. For example, a phenylalanine residue within the A.beta. sequence (such as Phe.sub.19 or Phe.sub.20) can be substituted with radioactive iodotyrosyl. Any of the various isotopes of radioactive iodine can be incorporated to create a diagnostic agent. Preferably, .sup.123 I (half-life=13.2 hours) is used for whole body scintigraphy, .sup.124 I (half life=4 days) is used for positron emission tomography (PET), .sup.125 I (half life=60 days) is used for metabolic turnover studies and .sup.131 I (half life=8 days) is used for whole body counting and delayed low resolution imaging studies.

Detailed Description Text (72):
Furthermore, an additional modification of a modulator compound of the invention can serve to confer an additional therapeutic property on the compound. That is, the additional chemical modification can comprise an additional functional moiety. For example, a functional moiety which serves to break down or dissolve amyloid plaques can be coupled to the modulator compound. In this form, the MG-ACD portion of the modulator serves to target the compound to A.beta. peptides and disrupt the polymerization of the A.beta. peptides, whereas the additional functional moiety serves to break down or dissolve amyloid plaques after the compound has been targeted to these sites.

Detailed Description Text (73):
In an alternative chemical modification, a .beta.-amyloid compound of the invention is prepared in a "prodrug" form, wherein the compound itself does not modulate A.beta. aggregation, but rather is capable of being transformed, upon metabolism in vivo, into a .beta.-amyloid modulator compound as defined herein. For example, in this type of compound, the modulating group can be present in a prodrug form that is capable of being converted upon metabolism into the form of an active modulating group. Such a prodrug form of a modifying group is referred to herein as a "secondary modifying group." A variety of strategies are known in the art for preparing peptide prodrugs that limit metabolism in order to optimize delivery of the active form of the peptide-based drug (see e.g., Moss, J. (1995) in Peptide-Based Drug Design: Controlling Transport and Metabolism, Taylor, M. D. and Amidon, G. L. (eds), Chapter 18. Additionally strategies have been specifically tailored to achieving CNS delivery based on "sequential metabolism" (see e.g., Bodor, N., et al. (1992) Science 257:1698-1700; Prokai, L., et al. (1994) J. Am. Chem. Soc. 116:2643-2644; Bodor, N. and Prokai, L. (1995) in Peptide-Based Drug Design: Controlling Transport and Metabolism, Taylor, M. D. and Amidon, G. L. (eds), Chapter 14. In one embodiment of a prodrug form of a modulator of the invention, the modifying group comprises an alkyl ester to facilitate blood-brain barrier permeability.

Detailed Description Text (74):
Modulator compounds of the invention can be prepared by standard techniques known in the art. The peptide component of a modulator can be synthesized using standard techniques such as those described in Bodansky, M. Principles of Peptide Synthesis, Springer Verlag, Berlin (1993) and Grant, G. A (ed.). Synthetic Peptides: A User's Guide, W. H. Freeman and Company, New York (1992). Automated peptide synthesizers are commercially available (e.g., Advanced ChemTech Model 396; Milligen/Biosearch 9600). Additionally, one or more modulating groups can be attached to the A.beta.-derived peptidic component (e.g., an A.beta. aggregation core domain) by standard methods, for example using methods for reaction through an amino group (e.g., the alpha-amino group at the amino-terminus of a peptide), a carboxyl group (e.g., at the carboxy terminus of a peptide), a hydroxyl group (e.g., on a tyrosine, serine or threonine residue) or other suitable reactive group on an amino acid side chain (see e.g., Greene, T. W and Wuts, P. G. M. Protective Groups in Organic Synthesis, John Wiley and Sons, Inc., New York (1991). Exemplary syntheses of D-amino acid .beta. amyloid modulator are described further in Example 1.

Detailed Description Text (76):
Another aspect of the invention pertains to a method for selecting a modulator of .beta.-amyloid aggregation. In the method, a test compound is contacted with natural .beta. amyloid peptides, the aggregation of the natural .beta.-AP is measured and a modulator is selected based on the ability of the test compound to alter the aggregation of the natural .beta.-AP (e.g., inhibit or promote aggregation). In a preferred embodiment, the test compound is contacted with a molar excess amount of the natural .beta.-AP. The amount and/or rate of natural .beta.-AP aggregation in the presence of the test compound can be determined by a suitable assay indicative of .beta.-AP aggregation, as described herein (see e.g., Example 2).

Detailed Description Text (77):
In a preferred assay, the natural .beta.-AP is dissolved in solution in the presence of the test compound and aggregation of the natural .beta.-AP is assessed in a nucleation assay (see Example 2) by assessing the turbidity of the solution over time, as measured by the apparent absorbance of the solution at 405 nm (described further in Example 2; see also Jarrett et al. (1993) Biochemistry 32:4693-4697). In the absence of a .beta.-amyloid modulator, the A.sub.405 nm of the solution typically stays relatively constant during a lag time in which the .beta.-AP remains in solution, but then the A.sub.405 nm of the solution rapidly increases as the .beta.-AP aggregates and comes out of solution, ultimately reaching a plateau level (i.e., the A.sub.405 nm of the solution exhibits sigmoidal kinetics over time). In contrast, in the presence of a test compound that inhibits .beta.-AP aggregation, the A.sub.405 nm of the solution is reduced compared to when the modulator is absent. Thus, in the presence of the inhibitory modulator, the solution may exhibit an increased lag time, a decreased slope of aggregation and/or a lower plateau level compared to when the modulator is absent. This method for selecting a modulator of .beta.-amyloid polymerization can similarly be used to select modulators that promote .beta.-AP aggregation. Thus, in the presence of a modulator that promotes .beta.-AP aggregation, the A.sub.405 nm of the solution is increased compared to when the modulator is absent (e.g., the solution may exhibit an decreased lag time, increase slope of aggregation and/or a higher plateau level compared to when the modulator is absent).

Detailed Description Text (78):
Another assay suitable for use in the screening method of the invention, a seeded extension assay, is also described further in Example 2. In this assay, .beta.-AP monomer and an aggregated .beta.-AP "seed" are combined, in the presence and absence of a test compound, and the amount of .beta.-fibril formation is assayed based on enhanced emission of the dye Thioflavine T when contacted with .beta.-AP fibrils. Moreover, .beta.-AP aggregation can be assessed by electron microscopy (EM) of the .beta.-AP preparation in the presence or absence of the modulator. For example, .beta. amyloid fibril formation, which is detectable by EM, is reduced in the presence of a modulator that inhibits .beta.-AP aggregation (i.e., there is a reduced amount or number of .beta.-fibrils in the presence of the modulator), whereas .beta. fibril formation is increased in the presence of a modulator that promotes .beta.-AP aggregation (i.e., there is an increased amount or number of .beta.-fibrils in the presence of the modulator).

Detailed Description Text (81):
Another aspect of the invention pertains to pharmaceutical compositions of the .beta.-amyloid modulator compounds of the invention. In one embodiment, the composition includes a .beta. amyloid modulator compound in a therapeutically or prophylactically effective amount sufficient to alter, and preferably inhibit, aggregation of natural .beta.-amyloid peptides, and a pharmaceutically acceptable carrier. In another embodiment, the composition includes a .beta. amyloid modulator compound in a therapeutically or prophylactically effective amount sufficient to inhibit the neurotoxicity of natural .beta.-amyloid peptides, and a pharmaceutically acceptable carrier. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as reduction or reversal or .beta.-amyloid deposition and/or reduction or reversal of A.beta. neurotoxicity. A therapeutically effective amount of modulator may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the modulator to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental effects of the modulator are outweighed by the therapeutically beneficial effects. The potential neurotoxicity of the modulators of the invention can be assayed using the cell-based assay described in Example 6 and a therapeutically effective modulator can be selected which does not exhibit significant neurotoxicity. In a preferred embodiment, a therapeutically effective amount of a modulator is sufficient to alter, and preferably inhibit, aggregation of a molar excess amount of natural .beta.-amyloid peptides. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, such as preventing or inhibiting the rate of .beta.-amyloid deposition and/or A.beta. neurotoxicity in a subject predisposed to .beta.-amyloid deposition. A prophylactically effective amount can be determined as described above for the therapeutically effective amount. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Detailed Description Text (82):
One factor that may be considered when determining a therapeutically or prophylactically effective amount of a .beta. amyloid modulator is the concentration of natural .beta.-AP in a biological compartment of a subject, such as in the cerebrospinal fluid (CSF) of the subject. The concentration of natural .beta.-AP in the CSF has been estimated at 3 nM (Schwartzman, (1994) Proc. Natl. Acad. Sci. USA 91:8368-8372). A non-limiting range for a therapeutically or prophylactically effective amounts of a .beta. amyloid modulator is 0.01 nM-10 .mu.M. It is to be noted that dosage values may vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

Detailed Description Text (83):
The amount of active compound in the composition may vary according to factors such as the disease state, age, sex, and weight of the individual, each of which may affect the amount of natural .beta.-AP in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be

proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

Detailed Description Text (85):
Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and gelatin. Moreover, the modulators can be administered in a time release formulation, for example in a composition which includes a slow release polymer. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). Many methods for the preparation of such formulations are patented or generally known to those skilled in the art.

Detailed Description Text (86):
Sterile injectable solutions can be prepared by incorporating the active compound (e.g., .beta.-amyloid modulator) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Detailed Description Text (87):
A modulator compound of the invention can be formulated with one or more additional compounds that enhance the solubility of the modulator compound. Preferred compounds to be added to formulations to enhance the solubility of the modulators are cyclodextrin derivatives, preferably hydroxypropyl-.gamma.-cyclodextrin. Drug delivery vehicles containing a cyclodextrin derivative for delivery of peptides to the central nervous system are described in Bodor, N., et al. (1992) Science 257:1698-1700. For the .beta.-amyloid modulators described herein, inclusion in the formulation of hydroxypropyl-.gamma.-cyclodextrin at a concentration 50-200 mM increases the aqueous solubility of the compounds. In addition to increased solubility, inclusion of a cyclodextrin derivative in the formulation may have other beneficial effects, since .beta.-cyclodextrin itself has been reported to interact with the A.beta. peptide and inhibit fibril formation in vitro (Camilleri, P., et al. (1994) FEBS Letters 341:256-258. Accordingly, use of a modulator compound of the invention in combination with a cyclodextrin derivative may result in greater inhibition of A.beta. aggregation than use of the modulator alone. Chemical modifications of cyclodextrins are known in the art (Hanessian, S., et al. (1995) J. Org. Chem. 60:4786-4797). In addition to use as an additive in a pharmaceutical composition containing a modulator of the invention, cyclodextrin derivatives may also be useful as modifying groups and, accordingly, may also be covalently coupled to an A.beta. peptide compound to form a modulator compound of the invention.

Detailed Description Text (89):

In another embodiment, a pharmaceutical composition comprising a modulator of the invention is formulated such that the modulator is transported across the blood-brain barrier (BBB). Various strategies known in the art for increasing transport across the BBB can be adapted to the modulators of the invention to thereby enhance transport of the modulators across the BBB (for reviews of such strategies, see e.g., Pardridge, W. M. (1994) Trends in Biotechnol. 12:239-245; Van Bree, J. B. et al. (1993) Pharm. World Sci. 15:2-9; and Pardridge, W. M. et al. (1992) Pharmacol. Toxicol. 71:3-10). In one approach, the modulator is chemically modified to form a prodrug with enhanced transmembrane transport. Suitable chemical modifications include covalent linking of a fatty acid to the modulator through an amide or ester linkage (see e.g., U.S. Pat. No. 4,933,324 and PCT Publication WO 89/07938, both by Shashoua; U.S. Pat. No. 5,284,876 by Hesse et al.; Toth, I. et al. (1994) J. Drug Target. 2:217-239; and Shashoua, V. E. et al. (1984) J. Med. Chem. 27:659-664) and glycating the modulator (see e.g., U.S. Pat. No. 5,260,308 by Poduslo et al.). Also, N-acylamino acid derivatives may be used in a modulator to form a "lipidic" prodrug (see e.g., U.S. Pat. No. 5,112,863 by Hashimoto et al.).

Detailed Description Text (90):
In another approach for enhancing transport across the BBB, a peptidic or peptidomimetic modulator is conjugated to a second peptide or protein, thereby forming a chimeric protein, wherein the second peptide or protein undergoes absorptive-mediated or receptor-mediated transcytosis through the BBB. Accordingly, by coupling the modulator to this second peptide or protein, the chimeric protein is transported across the BBB. The second peptide or protein can be a ligand for a brain capillary endothelial cell receptor ligand. For example, a preferred ligand is a monoclonal antibody that specifically binds to the transferrin receptor on brain capillary endothelial cells (see e.g., U.S. Pat. Nos. 5,182,107 and 5,154,924 and PCT Publications WO 93/10819 and WO 95/02421, all by Friden et al.). Other suitable peptides or proteins that can mediate transport across the BBB include histones (see e.g., U.S. Pat. No. 4,902,505 by Pardridge and Schimmel) and ligands such as biotin, folate, niacin, pantothenic acid, riboflavin, thiamin, pryridoxal and ascorbic acid (see e.g., U.S. Pat. Nos. 5,416,016 and 5,108,921, both by Heinstein). Additionally, the glucose transporter GLUT-1 has been reported to transport glycopeptides (L-serinyl-.beta.-D-glucoside analogues of [Met5]enkephalin) across the BBB (Polt, R. et al. (1994) Proc. Natl. Acad. Sci. USA 91:7114-1778). Accordingly, a modulator compound can be coupled to such a glycopeptide to target the modulator to the GLUT-1 glucose transporter. For example, a modulator compound which is modified at its amino terminus with the modifying group Aic (3-(O-aminoethyl-iso)-cholyl, a derivative of cholic acid having a free amino group) can be coupled to a glycopeptide through the amino group of Aic by standard methods. Chimeric proteins can be formed by recombinant DNA methods (e.g., by formation of a chimeric gene encoding a fusion protein) or by chemical crosslinking of the modulator to the second peptide or protein to form a chimeric protein. Numerous chemical crosslinking agents are known in the (e.g., commercially available from Pierce, Rockford Ill.). A crosslinking agent can be chosen which allows for high yield coupling of the modulator to the second peptide or protein and for subsequent cleavage of the linker to release bioactive modulator. For example, a biotin-avidin-based linker system may be used.

Detailed Description Text (95):
In another embodiment, a pharmaceutical composition of the invention is provided as a packaged formulation. The packaged formulation may include a pharmaceutical composition of the invention in a container and printed instructions for administration of the composition for treating a subject having a disorder associated with .beta.-amyloidosis, e.g. Alzheimer's disease.

Detailed Description Text (97):
Another aspect of the invention pertains to methods for altering the aggregation or inhibiting the neurotoxicity of natural .beta.-amyloid peptides. In the methods of the invention, natural .beta. amyloid peptides are contacted with a .beta. amyloid modulator such that the aggregation of the natural .beta. amyloid peptides is altered or the neurotoxicity of the natural .beta. amyloid peptides is inhibited. In a preferred embodiment, the modulator inhibits aggregation of the natural .beta. amyloid peptides. In another embodiment, the modulator promotes aggregation of the natural .beta. amyloid peptides. Preferably, aggregation of a molar excess amount of .beta.-AP, relative to the amount of modulator, is altered upon contact with the modulator.

Detailed Description Text (98):
In the method of the invention, natural .beta. amyloid peptides can be contacted with a modulator either in vitro or in vivo. Thus, the term "contacted with" is intended to

encompass both incubation of a modulator with a natural .beta.-AP preparation in vitro and delivery of the modulator to a site in vivo where natural .beta.-AP is present. Since the modulator compound interacts with natural .beta.-AP, the modulator compounds can be used to detect natural .beta.-AP, either in vitro or in vivo. Accordingly, one use of the modulator compounds of the invention is as diagnostic agents to detect the presence of natural .beta.-AP, either in a biological sample or in vivo in a subject. Furthermore, detection of natural .beta.-AP utilizing a modulator compound of the invention further can be used to diagnose amyloidosis in a subject. Additionally, since the modulator compounds of the invention disrupt .beta.-AP aggregation and inhibit .beta.-AP neurotoxicity, the modulator compounds also are useful in the treatment of disorders associated with .beta.-amyloidosis, either prophylactically or therapeutically. Accordingly, another use of the modulator compounds of the invention is as therapeutic agents to alter aggregation and/or neurotoxicity of natural .beta.-AP.

Detailed Description Text (99):
In one embodiment, a modulator compound of the invention is used in vitro, for example to detect and quantitate natural .beta.-AP in sample (e.g., a sample of biological fluid). To aid in detection, the modulator compound can be modified with a detectable substance. The source of natural .beta.-AP used in the method can be, for example, a sample of cerebrospinal fluid (e.g., from an AD patient, an adult susceptible to AD due to family history, or a normal adult). The natural .beta.-AP sample is contacted with a modulator of the invention and aggregation of the .beta.-AP is measured, such as by the assays described in Example 2. The degree of aggregation of the .beta.-AP sample can then be compared to that of a control sample(s) of a known concentration of .beta.-AP, similarly contacted with the modulator and the results can be used as an indication of whether a subject is susceptible to or has a disorder associated with .beta.-amyloidosis. Moreover, .beta.-AP can be detected by detecting a modulating group incorporated into the modulator. For example, modulators incorporating a biotin compound as described herein (e.g., an amino-terminally biotinylated .beta.-AP peptide) can be detected using a probe which or avidin probe which is labeled with a detectable substance (e.g., an enzyme, such as peroxidase).

Detailed Description Text (100):
In another embodiment, a modulator compound of the invention is used in vivo to detect, and, if desired, quantitate, natural .beta.-AP deposition in a subject, for example to aid in the diagnosis of .beta. amyloidosis in the subject. To aid in detection, the modulator compound can be modified with a detectable substance, preferably .sup.99m Tc or radioactive iodine (described further above), which can be detected in vivo in a subject. The labeled .beta.-amyloid modulator compound is administered to the subject and, after sufficient time to allow accumulation of the modulator at sites of amyloid deposition, the labeled modulator compound is detected by standard imaging techniques. The radioactive signal generated by the labeled compound can be directly detected (e.g., whole body counting), or alternatively, the radioactive signal can be converted into an image on an autoradiograph or on a computer screen to allow for imaging of amyloid deposits in the subject. Methods for imaging amyloidosis using radiolabeled proteins are known in the art. For example, serum amyloid P component (SAP), radiolabeled with either .sup.123 I or .sup.99m Tc, has been used to image systemic amyloidosis (see e.g., Hawkins, P. N. and Pepys, M. B. (1995) Eur. J. Nucl. Med. 22:595-599). Of the various isotypes of radioactive iodine, preferably .sup.123 I (half-life=13.2 hours) is used for whole body scintigraphy, 124I (half life=4 days) is used for positron emission tomography (PET), .sup.125 I (half life=60 days) is used for metabolic turnover studies and .sup.131 I (half life=8 days) is used for whole body counting and delayed low resolution imaging studies. Analogous to studies using radiolabeled SAP, a labeled modulator compound of the invention can be delivered to a subject by an appropriate route (e.g., intravenously, intraspinally, intracerebrally) in a single bolus, for example containing 100 .mu.g of labeled compound carrying approximately 180 MBq of radioactivity.

Detailed Description Text (101):
The invention provides a method for detecting the presence or absence of natural .beta.-amyloid peptides in a biological sample, comprising contacting a biological sample with a compound of the invention and detecting the compound bound to natural .beta.-amyloid peptides to thereby detect the presence or absence of natural .beta.-amyloid peptides in the biological sample. In one embodiment, the .beta.-amyloid modulator compound and the biological sample are contacted in vitro. In another embodiment, the .beta.-amyloid modulator compound is contacted with the biological sample by administering the .beta.-amyloid modulator compound to a subject. For in vivo administration, preferably the compound is labeled with radioactive technetium or

radioactive iodine.

Detailed Description Text (102):
The invention also provides a method for detecting natural .beta.-amyloid peptides to facilitate diagnosis of a .beta.-amyloidogenic disease, comprising contacting a biological sample with the compound of the invention and detecting the compound bound to natural .beta.-amyloid peptides to facilitate diagnosis of a .beta.-amyloidogenic disease. In one embodiment, the .beta.-amyloid modulator compound and the biological sample are contacted in vitro. In another embodiment, the .beta.-amyloid modulator compound is contacted with the biological sample by administering the .beta.-amyloid modulator compound to a subject. For in vivo administration, preferably the compound is labeled with radioactive technetium or radioactive iodine. Preferably, use of the method facilitates diagnosis of Alzheimer's disease.

Detailed Description Text (103):
In another embodiment, the invention provides a method for altering natural .beta.-AP aggregation or inhibiting .beta.-AP neurotoxicity, which can be used prophylactically or therapeutically in the treatment or prevention of disorders associated with .beta. amyloidosis, e.g., Alzheimer's Disease. Modulator compounds of the invention can reduce the toxicity of natural .beta.-AP aggregates to cultured neuronal cells. Moreover, the modulators also have the ability to reduce the neurotoxicity of preformed A.beta. fibrils. Accordingly, the modulator compounds of the invention can be used to inhibit or prevent the formation of neurotoxic A.beta. fibrils in subjects (e.g., prophylactically in a subject predisposed to .beta.-amyloid deposition) and can be used to reverse .beta.-amyloidosis therapeutically in subjects already exhibiting .beta.-amyloid deposition.

Detailed Description Text (104):
A modulator of the invention is contacted with natural .beta. amyloid peptides present in a subject (e.g., in the cerebrospinal fluid or cerebrum of the subject) to thereby alter the aggregation of the natural .beta.-AP and/or inhibit the neurotoxicity of the natural .beta.-APs. A modulator compound alone can be administered to the subject, or alternatively, the modulator compound can be administered in combination with other therapeutically active agents (e.g., as discussed above in subsection IV). When combination therapy is employed, the therapeutic agents can be coadministered in a single pharmaceutical composition, coadministered in separate pharmaceutical compositions or administered sequentially.

Detailed Description Text (105):
The modulator may be administered to a subject by any suitable route effective for inhibiting natural .beta.-AP aggregation in the subject, although in a particularly preferred embodiment, the modulator is administered parenterally, most preferably to the central nervous system of the subject. Possible routes of CNS administration include intraspinal administration and intracerebral administration (e.g., intracerebrovascular administration). Alternatively, the compound can be administered, for example, orally, intraperitoneally, intravenously or intramuscularly. For non-CNS administration routes, the compound can be administered in a formulation which allows for transport across the BBB. Certain modulators may be transported across the BBB without any additional further modification whereas others may need further modification as described above in subsection IV.

Detailed Description Text (107):
The method of the invention for altering .beta.-AP aggregation in vivo, and in particular for inhibiting .beta.-AP aggregation, can be used therapeutically in diseases associated with abnormal .beta. amyloid aggregation and deposition to thereby slow the rate of .beta. amyloid deposition and/or lessen the degree of .beta. amyloid deposition, thereby ameliorating the course of the disease. In a preferred embodiment, the method is used to treat Alzheimer's disease (e.g., sporadic or familial AD, including both individuals exhibiting symptoms of AD and individuals susceptible to familial AD). The method can also be used prophylactically or therapeutically to treat other clinical occurrences of .beta. amyloid deposition, such as in Down's syndrome individuals and in patients with hereditary cerebral hemorrhage with amyloidosis-Dutch-type (HCHWA-D). While inhibition of .beta.-AP aggregation is a preferred therapeutic method, modulators that promote .beta.-AP aggregation may also be useful therapeutically by allowing for the sequestration of .beta.-AP at sites that do not lead to neurological impairment.

Detailed Description Text (108):
Additionally, abnormal accumulation of .beta.-amyloid precursor protein in muscle

fibers has been implicated in the pathology of sporadic inclusion body myositis (IBM) (Askana, V. et al. (1996) Proc. Natl. Acad. Sci. USA 93:1314-1319; Askanas, V. et al. (1995) Current Opinion in Rheumatology 7:486-496). Accordingly, the modulators of the invention can be used prophylactically or therapeutically in the treatment of disorders in which .beta.-AP, or APP, is abnormally deposited at non-neurological locations, such as treatment of IBM by delivery of the modulators to muscle fibers.

Detailed Description Text (109):
This invention is further illustrated by the following examples which should not be construed as limiting. A modulator's ability to alter the aggregation of natural .beta.-amyloid peptide and/or inhibit the neurotoxicity of natural .beta.-amyloid peptide in the assays described below are predictive of the modulator's ability to perform the same function in vivo. The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated by reference.

Detailed Description Text (111):
Preparation of .beta.-Amyloid Modulator Compounds Comprising D-Amino Acids

Detailed Description Text (112):
.beta.-amyloid modulators comprising D-amino acids can be prepared by solid-phase peptide synthesis, for example using an N.sup..alpha. -9-fluorenylmethyloxycarbonyl (FMOC)-based protection strategy as follows. Starting with 2.5 mmoles of FMOC-D-Val-Wang resin, sequential additions of each amino acid are performed using a four-fold excess of protected amino acids, 1-hydroxybenzotriazole (HOBt) and diisopropyl carbodiimide (DIC). Recouplings are performed when necessary as determined by ninhydrin testing of the resin after coupling. Each synthesis cycle is minimally described by a three minute deprotection (25% piperidine/N-methyl-pyrrolidone (NMP)), a 15 minute deprotection, five one minute NMP washes, a 60 minute coupling cycle, five NMP washes and a ninhydrin test. For N-terminal modification, an N-terminal modifying reagent is substituted for an FMOC-D-amino acid and coupled to a 700 mg portion of the fully assembled peptide-resin by the above protocol. The peptide is removed from the resin by treatment with trifluoroacetic acid (TFA) (82.5%), water (5%), thioanisole (5%), phenol (5%), ethanedithiol (2.5%) for two hours followed by precipitation of the peptide in cold ether. The solid is pelleted by centrifugation (2400 rpm.times.10 min.), and the ether decanted. The solid is resuspended in ether, pelleted and decanted a second time. The solid is dissolved in 10% acetic acid and lyophilized to dryness. For structural analysis, 60 mg of the solid is dissolved in 25% acetonitrile (ACN)/0.1% TFA and applied to a C18 reversed phase high performance liquid chromatography (HPLC) column.

Detailed Description Text (113):
Alternatively, .beta.-amyloid modulators comprising D-amino acids can be prepared on an Advanced ChemTech Model 396 multiple peptide synthesizer using an automated protocol established by the manufacturer for 0.025 mmole scale synthesis. Double couplings are performed on all cycles using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)/N,N-diisopropylethylamine (DIEA)/HOBt/FMOC-D-amino acid in four-fold excess for 30 minutes followed by DIC/HOBt/FMOC-D-amino acid in four-fold excess for 45 minutes. The peptide is deprotected and removed from the resin by treatment with TFA/water (95%/5%) for three hours and precipitated with ether as described above. The pellet is resuspended in 10% acetic acid and lyophilized. The material is purified by a preparative HPLC using 15%-40% acetonitrile over 80 minutes on a Vydac C18 column (21.times.250 mm).

Detailed Description Text (114):
Various N-terminally modified .beta.-amyloid modulator compounds can be synthesized using standard methods. Fully-protected resin-bound peptides are prepared as described above on Wang resin to eventually afford carboxyl terminal peptide acids. Small portions of each peptide resin (e.g., 13-20 .mu.moles) are aliquoted into the wells of the reaction block of an Advanced ChemTech Model 396 Multiple Peptide Synthesizer. The N-terminal FMOC protecting group of each sample is removed in the standard manner with 25% piperidine in NMP followed by extensive washing with NMP. The unprotected N-terminal .alpha.-amino group of each peptide-resin sample can be modified using one of the following methods:

Detailed Description Text (115):
Method A, coupling of modifying reagents containing free carboxylic acid groups: The modifying reagent (five equivalents) is predissolved in NMP, DMSO or a mixture of these two solvents. HOBT and DIC (five equivalents of each reagent) are added to the

dissolved modifier and the resulting solution is added to one equivalent of free-amino peptide-resin. Coupling is allowed to proceed overnight, followed by washing. If a ninhydrin test on a small sample of peptide-resin shows that coupling is not complete, the coupling is repeated using 1-hydroxy-7-azabenzotriazole (HOAt) in place of HOBt.

Detailed Description Text (119):
.beta.-Amyloid Aggregation Assays

Detailed Description Text (120):
The ability of .beta.-amyloid modulator compounds to modulate (e.g., inhibit or promote) the aggregation of natural .beta.-AP when combined with the natural .beta.-AP can be examined in one or both of the aggregation assays described below. Natural .beta.-AP (.beta.-AP.sub.1-40) for use in the aggregation assays is commercially available from Bachem (Torrance, Calif.).

Detailed Description Text (122):
The nucleation assay is employed to determine the ability of test compounds to alter (e.g. inhibit) the early events in formation of .beta.-AP fibers from monomeric .beta.-AP. Characteristic of a nucleated polymerization mechanism, a lag time is observed prior to nucleation, after which the peptide rapidly forms fibers as reflected in a linear rise in turbidity. The time delay before polymerization of .beta.-AP monomer can be quantified as well as the extent of formation of insoluble fiber by light scattering (turbidity). Polymerization reaches equilibrium when the maximum turbidity reaches a plateau. The turbidity of a solution of natural .beta.-AP in the absence or presence of various concentrations of a .beta.-amyloid modulator compound is determined by measuring the apparent absorbance of the solution at 405 nm (A.sub.405 nm) over time. The threshold of sensitivity for the measurement of turbidity is in the range of 15-20 .mu.M .beta.-AP. A decrease in turbidity over time in the presence of the modulator, as compared to the turbidity in the absence of the modulator, indicates that the modulator inhibits formation of .beta.-AP fibers from monomeric .beta.-AP. This assay can be performed using stirring or shaking to accelerate polymerization, thereby increasing the speed of the assay. Moreover the assay can be adapted to a 96-well plate format to screen multiple compounds.

Detailed Description Text (124):
.beta.-amyloid aggregation in the absence of any modulators results in enhanced turbidity of the natural .beta.-AP solution (i.e., an increase in the apparent absorbance at 405 nm over time). Accordingly, a solution including an effective inhibitory modulator compound exhibits reduced turbidity as compared to the control sample without the modulator compound (i.e., less apparent absorbance at 405 nm over time as compared to the control sample).

Detailed Description Text (125):
Alternative to use of turbidity to quantitate .beta.-amyloid aggregation, fluorescence of thioflavin T (Th-T) also can be used to quantitate .beta.-amyloid aggregation in the nucleation assay (use of Th-T fluorescence for quantitating .beta.-amyloid aggregation is described further below for the seeded extension assay).

Detailed Description Text (127):
The seeded extension assay can be employed to measure the rate of A.beta. fiber formed in a solution of A.beta. monomer following addition of polymeric A.beta. fiber "seed". The ability of test compounds to prevent further deposition of monomeric A.beta. to previously deposited amyloid is determined using a direct indicator of .beta.-sheet formation using fluorescence. In contrast with the nucleation assay, the addition of seed provides immediate nucleation and continued growth of preformed fibrils without the need for continuous mixing, and thus results in the absence of a lag time before polymerization starts. Since this assay uses static polymerization conditions, the activity of positive compounds in the nucleation assay can be confirmed in this second assay under different conditions and with an additional probe of amyloid structure.

Detailed Description Text (128):
In the seeded extension assay, monomeric A.beta..sub.1-40 is incubated in the presence of a "seed" nucleus (approximately ten mole percent of A.beta. that has been previously allowed to polymerize under controlled static conditions). Samples of the solution are then diluted in thioflavin T (Th-T). The polymer-specific association of Th-T with A.beta. produces a fluorescent complex that allows the measurement of the extent of fibril formation (Levine, H. (1993) Protein Science 2:404-410). In particular, association of Th-T with aggregated .beta.-AP, but not monomeric or loosely associated .beta.-AP, gives rise to a new excitation (ex) maximum at 450 nm and an enhanced

emission (em) at 482 nm, compared to the 385 nm (ex) and 445 nm (em) for the free dye. Small aliquots of the polymerization mixture contain sufficient fibril to be mixed with Th-T to allow the monitoring of the reaction mixture by repeated sampling. A linear growth curve is observed in the presence of excess monomer. The formation of thioflavin T responsive .beta.-sheet fibrils parallels the increase in turbidity observed using the nucleation assay.

Detailed Description Text (131):
.beta.-amyloid aggregation results in enhanced emission of Thioflavin-T. Accordingly, samples including an effective inhibitory modulator compound exhibit reduced emission as compared to control samples without the modulator compound.

Detailed Description Text (133):
Analysis of .beta.-Amyloid Modulator Compounds Comprising D-Amino Acids

Detailed Description Text (134):
In this example, D-amino acid-containing modulator compounds designed based upon the A.beta. aggregation core domain A.beta..sub.17-21 were prepared and tested for their ability to inhibit aggregation of natural .beta.-amyloid peptide using aggregations assays as described in Example 2. Abbreviations used in this example are: h- (free amino terminus), -oh (free carboxylic acid terminus), -nh.sub.2 (amide terminus), CA (cholyl, the acyl portion of cholic acid), PEA (phenethylamide) and d (D-amino acid). Compounds in which the amino acid residues are in parentheses and preceded by "d" indicate that all amino acid residues are D-amino acids. For example, d(LVFFA) indicates D-Leu-D-Val-D-Phe-D-Phe-D-Ala.

Detailed Description Text (136):
The results shown in Table I demonstrate that all D-amino acid-containing modulators designed based on the A.beta..sub.17-21 region are effective inhibitors of A.beta. aggregation. Effective inhibitors can comprise, for example, all D-amino acid compounds corresponding to the entire A.beta..sub.17-21 region (e.g., PPI-457), to a smaller portion thereof (e.g., PPI-458, comprising A.beta..sub.17-20) or to a rearranged sequence thereof (e.g., PPI-454). The carboxy terminus of effective inhibitors can comprise, for example, a free carboxylic acid terminus (e.g., PPI-454) or a C-terminal amide modification (e.g., PPI-457 and PPI-458).

Detailed Description Text (137):
In a second series of experiments using all D-amino acid modulators, a different stock of A.beta..sub.1-40 was used in the nucleation assays from that used for the experiments shown in Table I. This new stock exhibited some delay in lag time even in absence of inhibitor and therefore the fold increase in lag time in the presence of test inhibitors was lower in these experiments compared to previous experiments. Despite this difference, the ability of a variety of all D-amino acid-containing modulators to inhibit A.beta. aggregation was evident compared to the negative control, an all D-alanine containing peptide (PPI-473). The results of this series of experiments, in which test compounds were assayed at 2, 3, 4 or 5 .mu.M, are shown below in Table II.

Detailed Description Text (138):
The results shown in Table II further demonstrate that all D-amino acid-containing modulators designed based on the A.beta..sub.17-21 region are effective inhibitors of A.beta. aggregation.

Detailed Description Text (140):
Variation of the N-Terminal Modifying Group on D-Amino Acid-Based Modulator Compounds

Detailed Description Text (141):
In this example, a series of modulator compounds were prepared which differed in their N-terminal modifying groups. The ability of the modulator compounds to inhibit aggregation of natural .beta.-amyloid peptide was evaluated using aggregations assays as described in Example 2. Abbreviations used in this example and presentation of the data are the same as described in Example 3. The results for compounds modified with N-terminal modifying groups derived from different bile acids are shown below in Table III. The results for compounds modified with various hydrophobic N-terminal modifying groups are shown below in Table IV. The results for compounds modified with various hydrophobic N-terminal modifying groups are shown below in Table IV. The results for compounds modified with various N-terminal hydroxylated and oxygenated modifying groups are shown below in Table V. Compounds exhibiting a change in lag time (.DELTA.Lag) of 1.3 or greater are highlighted in bold.

Detailed Description Text (144):
D-Amino Acid-Based Modulator Compounds Having a Free Amino-Terminus

Detailed Description Text (145):
In this example, the necessity for an N-terminal modifying group on the D-amino acid-based modulator compounds was evaluated. Peptides comprised entirely of D-amino acids and having a free amino terminus were prepared and tested for their ability to inhibit aggregation of natural .beta.-amyloid peptide using aggregations assays as described in Example 2. Abbreviations used in this example and presentation of the data are the same as described in Example 3. The results are shown below in Table VI. Compounds exhibiting a change in lag time (.DELTA.Lag) of 1.3 or greater are highlighted in bold.

Detailed Description Text (146):
The results shown in Table VI demonstrate that modulators comprising all D-amino acids and having a free amino terminus are effective at inhibiting aggregation of natural .beta.-amyloid peptides (i.e., an N-terminal modifying group is not required for the D-amino acid-containing modulators to effectively inhibit aggregation of natural .beta.-amyloid peptides). A particularly preferred D-amino acid modulator compound having a free amino-terminus is PPI-579, the retro-inverso isomer of A.beta..sub.17-21 (A.sub.21 .fwdarw.F) with a C-terminal amide.

Detailed Description Text (149):
The neurotoxicity of natural .beta.-amyloid peptide aggregates, in either the presence or absence of a .beta.-amyloid modulator, can be tested in a cell-based assay using either a rat or human neuronally-derived cell line (PC-12 cells or NT-2 cells, respectively) and the viability indicator 3,(4,4-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT). (See e.g., Shearman, M. S. et al. (1994) Proc. Natl. Acad. Sci. USA 91:1470-1474; Hansen, M. B. et al. (1989) J. Immun. Methods 119:203-210 for a description of similar cell-based viability assays). PC-12 is a rat adrenal pheochromocytoma cell line and is available from the American Type Culture Collection, Rockville, Md. (ATCC CRL 1721). MTT (commercially available from Sigma Chemical Co.) is a chromogenic substrate that is converted from yellow to blue in viable cells, which can be detected spectrophotometrically.

Detailed Description Text (150):
To test the neurotoxicity of natural .beta.-amyloid peptides, stock solutions of fresh A.beta. monomers and aged A.beta. aggregates are first prepared. A.beta..sub.1-40 in 100% DMSO is prepared from lyophilized powder and immediately diluted in one half the final volume in H.sub.2 O and then one half the final volume in 2.times. PBS so that a final concentration of 200 .mu.M peptide, 4% DMSO is achieved. Peptide prepared in this way and tested immediately on cells is referred to as "fresh" A.beta. monomer. To prepare "aged" A.beta. aggregates, peptide solution is placed in a 1.5 ml Eppendorf tube and incubated at 37.degree. C. for eight days to allow fibrils to form. Such "aged" A.beta. peptide can be tested directly on cells or frozen at -80.degree. C. The neurotoxicity of fresh monomers and aged aggregates are tested using PC12 and NT2 cells. PC12 cells are routinely cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% horse serum, 5% fetal calf serum, 4 mM glutamine, and 1% gentamycin. NT2 cells are routinely cultured in OPTI-MEM medium (GIBCO BRL CAT. #31985) supplemented with 10% fetal calf serum, 2 mM glutamine and 1% gentamycin. Cells are plated at 10-15,000 cells per well in 90 .mu.l of fresh medium in a 96-well tissue culture plate 3-4 hours prior to treatment. The fresh or aged A.beta. peptide solutions (10 .mu.L) are then diluted 1:10 directly into tissue culture medium so that the final concentration is in the range of 1-10 .mu.M peptide. Cells are incubated in the presence of peptide without a change in media for 48 hours at 37.degree. C. For the final three hours of exposure of the cells to the .beta.-AP preparation, MTT is added to the media to a final concentration of 1 mg/ml and incubation is continued at 37.degree. C. Following the two hour incubation with MTT, the media is removed and the cells are lysed in 100 .mu.L isopropanol/0.4N HCl with agitation. An equal volume of PBS is added to each well and the plates are agitated for an additional 10 minutes. Absorbance of each well at 570 nm is measured using a microtiter plate reader to quantitate viable cell.

Detailed Description Text (152):
To determine the effect of a .beta.-amyloid modulator compound on the neurotoxicity of A.beta..sub.1-40 aggregates, a modulator compound is preincubated with A.beta..sub.1-40 monomers under standard nucleation assay conditions as described in Example 2 and at

particular time intervals post-incubation, aliquots of the .beta.-AP/modulator solution are removed and 1) the turbidity of the solution is assessed as a measure of aggregation and 2) the solution is applied to cultured neuronal cells for 48 hours at which time cell viability is assessed using MTT to determine the neurotoxicity of the solution. Additionally, the ability of .beta.-amyloid modulator compounds to reduce the neurotoxicity of preformed A.beta..sub.1-40 aggregates can be assayed. In these experiments, A.beta..sub.1-40 aggregates are preformed by incubation of the monomers in the absence of any modulators. The modulator compound is then incubated with the preformed A.beta..sub.1-40 aggregates for 24 hours at 37.degree. C., after which time the .beta.-AP/modulator solution is collected and its neurotoxicity evaluated as described above.

Detailed Description Text (157):
A: 0.1% Trifluoroacetic acid (TFA) in water (v/v)

Detailed Description Text (163):
The above-described CSF stability assay was used to compare the CSF stability of a L-amino acid-based modulator compound (PPI-368, having the structure cholyl-Leu-Val-Phe-Phe-Ala-OH SEQ ID NO: 34) with an analogous D-amino acid-based peptide acid (PPI-433, having the structure cholyl-D-Leu-D-Val-D-Phe-D-Phe-D-Ala-OH) and an analogous D-amino acid-based peptide amide (PPI-457, having the structure cholyl-D-Leu-D-Val-D-Phe-D-Phe-D-Ala-NH.sub.2). The results, summarized in the bar graph shown in FIG. 1, demonstrate that both D-amino acid-based compounds exhibit significantly greater stability in CSF than the L-amino acid-based compound.

Detailed Description Text (167):
The above-described assay was used to measure the brain uptake of four cholyl-modified modulator compounds: PPI-382 (having the structure cholyl-Leu-Val-Phe-Phe-Ala-NH.sub.2) (SEQ ID NO: 33), PPI-457 (having the structure cholyl-D-Leu-D-Val-D-Phe-D-Phe-D-Ala-NH.sub.2), PPI-458 (having the structure cholyl-D-Leu-D-Val-D-Phe-D-Phe-NH.sub.2) and PPI-494 (having the structure cholyl-D-Leu-D-Val-D-Phe-phenethylamide). Radiolabel was introduced into the test compounds by using .sup.14 C-labelled cholic acid for modification. The vehicle used for the test compounds was 50 mM cyclodextrin in 75% phosphate buffered saline. Water was used as the freely diffusable reference, sucrose was used as a negative control and cholic acid was used as a control for the diffusability of the modifying group. The results are summarized below in Table VII.

Detailed Description Text (168):
The results indicate that the D-amino acid-based compounds (PPI-457, PPI-458 and PPI-494) exhibited greater brain uptake than the L-amino acid-based compound (PPI-382). An acetyl-modified D-amino acid based compound (PPI-472, having the structure acetyl-D-Leu-D-Val-D-Phe-D-Phe-NH.sub.2) exhibited a similar brain uptake index as PPI-458 and PPI-494 (i.e., about 4.5).

Detailed Description Paragraph Table (1):
Modifying Group Modifying Reagent
Cholyl- Cholic acid Lithocholyl- Lithocholic acid Hyodeoxycholyl- Hyodeoxycholic acid Chenodeoxycholyl- Chenodeoxycholic acid Ursodeoxycholyl- Ursodeoxycholic acid 3-Hydroxycinnamoyl- 3-Hydroxycinnamic acid 4-Hydroxycinnamoyl- 4-Hydroxycinnamic acid 2-Hydroxycinnamoyl- 2-Hydroxycinnamic acid 3-Hydroxy-4-methoxycinnamoyl- 3-Hydroxy-4-methoxycinnamic acid 4-Hydroxy-3-methoxycinnamoyl- 4-Hydroxy-3-methoxycinnamic acid 2-Carboxycinnamoyl- 2-Carboxycinnamic acid 3-Formylbenzoyl 3-Carboxybenzaldehyde 4-Formylbenzoyl 4-Carboxybenzaldehyde 3,4,-Dihydroxyhydrocinnamoyl- 3,4,-Dihydroxyhydrocinnamic acid 3,7-Dihydroxy-2-napthoyl- 3,7-Dihydroxy-2-naphthoic acid 4-Formylcinnamoyl- 4-Formylcinnamic acid 2-Formylphenoxyacetyl- 2-Formylphenoxyacetic acid 8-Formyl-1-napthoyl 1,8-napthaldehydic acid 4-(hydroxymethyl)benzoyl- 4-(hydroxymethyl)benzoic acid 4-Hydroxyphenylacetyl- 4-Hydroxyphenylacetic acid 3-Hydroxybenzoyl- 3-Hydroxybenzoic acid 4-Hydroxybenzoyl- 4-Hydroxybenzoic acid 5-Hydantoinacetyl- 5-Hydantoinacetic acid L-Hydroorotyl- L-Hydroorotic acid 4-Methylvaleryl- 4-Methylvaleric acid 2,4-Dihydroxybenzoyl- 2,4-Dihydroxybenzoic acid 3,4-Dihydroxycinnamoyl- 3,4-Dihydroxycinnamic acid 3,5-Dihydroxy-2-naphthoyl- 3,5-Dihydroxy-2-naphthoic acid 3-Benzoylpropanoyl- 3-Benzoylpropanoic acid trans-Cinnamoyl- trans-Cinnamic acid Phenylacetyl- Phenylacetic acid Diphenylacetyl- Diphenylacetic acid Triphenylacetyl- Triphenylacetic acid 2-Hydroxyphenylacetyl- 2-Hydroxyphenylacetic acid 3-Hydroxyphenylacetyl- 3-Hydroxyphenylacetic acid 4-Hydroxyphenylacetyl- 4-Hydroxyphenylacetic acid (.+-.)-Mandelyl- (.+-.)-Mandelic acid (.+-.)-2,4-Dihydroxy-3,3- (.+-.)-Pantolactone dimethylbutanoyl Butanoyl- Butanoic

anhydride Isobutanoyl- Isobutanoic anhydride Hexanoyl- Hexanoic anhydride Propionyl- Propionic anhydride 3-Hydroxybutyroyl .beta.-Butyrolactone 4-Hydroxybutyroyl .gamma.-Butyrolactone 3-Hydroxypropionoyl .alpha.-Propiolactone 2,4-Dihydroxybutyroyl 1-Hydroxy-.beta.-Butyrolactone 1-Adamantanecarbonyl- 1-Adamantanecarbonic acid Glycolyl- Glycolic acid DL-3-(4-hydroxyphenyl)lactyl- DL-3-(4-hydroxyphenyl)lactic acid 3-(2-Hydroxyphenyl)propionyl- 3-(2-Hydroxyphenyl)propionic acid 4-(2-Hydroxyphenyl)propionyl- 4-(2-Hydroxyphenyl)propionic acid D-3-Phenyllactyl- D-3-Phenyllactic acid Hydrocinnamoyl- Hydrocinnamic acid 3-(4-Hydroxyphenyl)propionyl- 3-(4-Hydroxyphenyl)propionic acid L-3-Phenyllactyl- L-3-Phenyllactic acid 4-methylvaleryl 4-methylvaleric acid 3-pyridylacetyl 3-pyridylacetic acid 4-pyridylacetyl 4-pyridylacetic acid Isonicotinoyl 4-quinolinecarboxyl 4-quinolinecarboxylic acid 1-isoquinolinecarboxyl 1-isoquinolinecarboxylic acid 3-isoquinolinecarboxyl 3-isoquinolinecarboxylic acid

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Detailed Description Paragraph Table (4):

TABLE III Modifying Groups Derived from Bile Acids C-Term. .DELTA.Lag Ref. # N-Term. Mod. Peptide Mod. 5 .mu.M PPI-424 Cholyl- LVFFA oh >6.0 (SEQ ID NO: 3) PPI-425 Lithocholyl- LVFFA oh 1.4 (SEQ ID NO: 3) PPI-520 Hyodeoxycholyl- LVFFA oh >2.3 (SEQ ID NO: 3) PPI-521 Chenodeoxycholyl- LVFFA oh >2.3 (SEQ ID NO: 3) PPI-522 Ursodeoxycholyl- LVFFA oh >2.3 (SEQ ID NO: 3)

Detailed Description Paragraph Table (8):

TABLE VII Compound Brain Uptake Index (.+-. SEM) Water 100 Sucrose 0.78 .+-. 0.05 Cholic Acid 1.02 .+-. 0.09 PPI-382 1.79 .+-. 0.04 PPI-457 3.09 .+-. 0.34 PPI-458 4.25 .+-. 0.49 PPI-494 4.78 .+-. 0.36

Detailed Description Paragraph Table (13):

SEQ ID NO: A.beta. Amino Acids Peptide Sequence 1 43 amino acids A.beta..sub.1-43 2 103 amino acids APP C-terminus 3 A.beta..sub.17-21 LVFFA 4 A.beta..sub.17-21 retro-inverso isomer d(AFFVL) 5 A.beta..sub.17-21 (V.sub.18 .fwdarw.L) retro-inverso d(AFFLL) 6 A.beta..sub.17-21 (A.sub.21 .fwdarw.L) retro-inverso d(LFFVL) 7 A.beta..sub.17-21 (A.sub.21 .fwdarw.F) retro-inverso d(FFFVL) 8 A.beta..sub.17-20 LVFF 9 A.beta..sub.17-20 inverso isomer d(LVFF) 10 A.beta..sub.17-19 inverso isomer (C-term. mod.) d(LVF)phenethyl- amide 11 A.beta..sub.17-20 (F.sub.19 .fwdarw.Y) inverso isomer d(LVYF) 12 A.beta..sub.17-26 (F.sub.19 .fwdarw.IodoY) inverso d(LV(IodoY)F) 13 A.beta..sub.17-20 (F.sub.20 .fwdarw.Y) inverso isomer d(LVFY) 14 A.beta..sub.17-20 (F.sub.20 .fwdarw.IodoY) inverso d(LVF(IodoY)) 15 A.beta..sub.17-20 (F.sub.20 .fwdarw.A) inverso isomer d(LVFA) 16 A.beta..sub.17-21 inverso isomer d(LVFFA) 17 A.beta..sub.17-21 (L.sub.17 .fwdarw.A; A.sub.21 .fwdarw.L) inverso isomer d(AVFFL) 18 A.beta..sub.17-21 (F.sub.19 .fwdarw.Y) inverso isomer d(LVYFA) 19 A.beta..sub.17-21 (F.sub.19 .fwdarw.IodoY) inverso d(LV(IodoY)FA) 20 A.beta..sub.17-21 (F.sub.20 .fwdarw.Y) inverso isomer d(LVFYA) 21 A.beta..sub.17-21 (F.sub.20 .fwdarw.IodoY) inverso d(LVF(IodoY)A) 22 A.beta..sub.17-20 retro-inverso isomer d(FFVL) 23 A.beta..sub.18-21 retro-inverso isomer d(AFFV) 24 A.beta..sub.17-21 (L.sub.17 .fwdarw.V; V.sub.18 .fwdarw.L; A.sub.21 .fwdarw.F) d(FFFLV) retro-inverso isomer 25 A.beta..sub.17-21 (V.sub.18 .fwdarw.F; A.sub.21 .fwdarw.F) d(FFFFL) retro-inverso isomer 26 A.beta..sub.17-21 (V.sub.18 .fwdarw.F) retro-inverso d(AFFFL) 27 A.beta..sub.17-21 (A.sub.21 .fwdarw.F) d(LVFFF) 28 A.beta..sub.17-21 (A.sub.21 .fwdarw.V) d(LVFFV) 29 A.beta..sub.17-21 (F.sub.19 .fwdarw.Y, F.sub.20 .fwdarw.Y) d(LVYYA) 30 A.beta..sub.17-21 (V.sub.18 .fwdarw.F, A.sub.21 .fwdarw.L) d(LFFFL) 31 N/A DDIIL-Adp 32 A.beta..sub.17-21 (F.sub.19 .fwdarw.Y) LVYFA 33 A.beta..sub.17-21 cholyl-LVFFA-amide 34 A.beta..sub.17-21 cholyl-LVFFA-OH

Detailed Description Paragraph Table (14):

SEQUENCE LISTING - (1) GENERAL INFORMATION: - (iii) NUMBER OF SEQUENCES: 34 - (2) INFORMATION FOR SEQ ID NO:1: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 43 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (v) FRAGMENT TYPE: internal - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1: - Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Gl - #u Val His His Gln Lys # 15 - Leu Val Phe Phe Ala Glu Asp Val Gly Ser As - #n Lys Gly Ala Ile Ile # 30 - Gly Leu Met Val Gly Gly Val Val Ile Ala Th - #r # 40 - (2) INFORMATION FOR SEQ ID NO:2: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 103 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (v) FRAGMENT TYPE: internal - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2: - Glu Val Lys Met Asp

Ala Glu Phe Arg His As - #p Ser Gly Tyr Glu Val # 15 - His His Gln Lys Leu Val Phe Phe Ala Glu As - #p Val Gly Ser Asn Lys # 30 - Gly Ala Ile Ile Gly Leu Met Val Gly Gly Va - #l Val Ile Ala Thr Val # 45 - Ile Val Ile Thr Leu Val Met Leu Lys Lys Ly - #s Gln Tyr Thr Ser Ile # 60 - His His Gly Val Val Glu Val Asp Ala Ala Va - #l Thr Pro Glu Glu Arg #80 - His Leu Ser Lys Met Gln Gln Asn Gly Tyr Gl - #u Asn Pro Thr Tyr Lys # 95 - Phe Phe Glu Gln Met Gln Asn 100 - (2) INFORMATION FOR SEQ ID NO:3: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3: - Leu Val Phe Phe Ala #5 - (2) INFORMATION FOR SEQ ID NO:4: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4: - Ala Phe Phe Val Leu #5 - (2) INFORMATION FOR SEQ ID NO:5: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5: - Ala Phe Phe Leu Leu #5 - (2) INFORMATION FOR SEQ ID NO:6: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6: - Leu Phe Phe Val Leu #5 - (2) INFORMATION FOR SEQ ID NO:7: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7: - Phe Phe Phe Val Leu #5 - (2) INFORMATION FOR SEQ ID NO:8: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 4 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8: - Leu Val Phe Phe - (2) INFORMATION FOR SEQ ID NO:9: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 4 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-4 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9: - Leu Val Phe Phe - (2) INFORMATION FOR SEQ ID NO:10: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 3 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-3 #/note= D amino acidINFORMATION: - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 3 #/note= phenethylamide C-terminal modification - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10: - Leu Val Phe - (2) INFORMATION FOR SEQ ID NO:11: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 4 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-4 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11: - Leu Val Tyr Phe - (2) INFORMATION FOR SEQ ID NO:12: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 4 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-4 #/note= D amino acidINFORMATION: - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 3 #/note= Xaa=iodotyrosineRMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12: - Leu Val Xaa Phe - (2) INFORMATION FOR SEQ ID NO:13: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 4 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-4 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13: - Leu Val Phe Tyr - (2) INFORMATION FOR SEQ ID NO:14: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 4 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-4 #/note= D amino acidINFORMATION: - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 4 #/note= Xaa=iodotyrosineRMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14: - Leu Val Phe Xaa - (2) INFORMATION FOR SEQ ID NO:15: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 4 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-4 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15: - Leu Val Phe Ala - (2) INFORMATION FOR SEQ ID NO:16: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16: - Leu Val Phe Phe Ala #5 - (2) INFORMATION FOR SEQ ID NO:17: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17: - Ala Val Phe Phe Leu #5 - (2) INFORMATION FOR SEQ ID NO:18: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18: - Leu Val Tyr Phe Ala #5 -

(2) INFORMATION FOR SEQ ID NO:19: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide

Detailed Description Paragraph Table (15):
- (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 3 #/note= Xaa=iodotyrosineRMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19: - Leu Val Xaa Phe Ala #5 - (2) INFORMATION FOR SEQ ID NO:20: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20: - Leu Val Phe Tyr Ala #5 - (2) INFORMATION FOR SEQ ID NO:21: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 4 #/note= Xaa=iodotyrosineRMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21: - Leu Val Phe Xaa Ala #5 - (2) INFORMATION FOR SEQ ID NO:22: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 4 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-4 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22: - Phe Phe Val Leu - (2) INFORMATION FOR SEQ ID NO:23: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 4 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-4 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23: - Ala Phe Phe Val - (2) INFORMATION FOR SEQ ID NO:24: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24: - Phe Phe Phe Leu Val #5 - (2) INFORMATION FOR SEQ ID NO:25: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25: - Phe Phe Phe Phe Leu #5 - (2) INFORMATION FOR SEQ ID NO:26: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26: - Ala Phe Phe Phe Leu #5 - (2) INFORMATION FOR SEQ ID NO:27: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27: - Leu Val Phe Phe Phe #5 - (2) INFORMATION FOR SEQ ID NO:28: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28: - Leu Val Phe Phe Val #5 - (2) INFORMATION FOR SEQ ID NO:29: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29: - Leu Val Tyr Tyr Ala #5 - (2) INFORMATION FOR SEQ ID NO:30: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1-5 #/note= D amino acidINFORMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30: - Leu Phe Phe Phe Leu #5 - (2) INFORMATION FOR SEQ ID NO:31: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 5 #/note= Adp modificationRMATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31: - Asp Asp Ile Ile Leu #5 - (2) INFORMATION FOR SEQ ID NO:32: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32: - Leu Val Tyr Phe Ala #5 - (2) INFORMATION FOR SEQ ID NO:33: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1 #/note= cholyl modificationTION: - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 5 #/note= amide modificationATION: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33: - Leu Val Phe Phe Ala #5 - (2) INFORMATION FOR SEQ ID NO:34: - (i) SEQUENCE CHARACTERISTICS: #acids (A) LENGTH: 5 amino (B) TYPE: amino acid (D) TOPOLOGY: linear - (ii) MOLECULE TYPE: peptide - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 1 #/note= cholyl modificationTION: - (ix) FEATURE: #site (A) NAME/KEY: Modified (B) LOCATION: 5 #/note= hydroxyl modificationON: - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34: - Leu Val Phe Phe Ala

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Weinreb, Paul H. et al (1994) "Peptide Models of a Hydrophobic Cluster at the C-Terminus of the .beta.-Amyloid Protein" Journal of the American Chemical Society 116(23) 10835-10836;.

CLAIMS:

10. A method for inhibiting aggregation of natural .beta.-amyloid peptides, comprising contacting the natural .beta.-amyloid peptides with the compound of claim 1 such that aggregation of the natural .beta.-amyloid peptides is inhibited.

11. A method for detecting the presence or absence of natural .beta.-amyloid peptides in a biological sample, comprising:

contacting a biological sample with the compound of claim 1, wherein the compound is labeled with a detectable substance; and

detecting the compound bound to natural .beta.-amyloid peptides to thereby detect the presence or absence of natural .beta.-amyloid peptides in the biological sample.

15. A method for treating a subject for a disorder associated with .beta.-amyloidosis, comprising:

administering to the subject a therapeutically effective amount of the compound of claim 1 such that the subject is treated for a disorder associated with .beta.-amyloidosis.

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DOCUMENT-IDENTIFIER: US 5854204 A
TITLE: A.beta. peptides that modulate .beta.-amyloid aggregation

Abstract Text (1):
Compounds that modulate the aggregation of amyloidogenic proteins or peptides are disclosed. The modulators of the invention can promote amyloid aggregation or, more preferably, can inhibit natural amyloid aggregation. In a preferred embodiment, the compounds modulate the aggregation of natural .beta. amyloid peptides (.beta.-AP). In a preferred embodiment, the .beta. amyloid modulator compounds of the invention are comprised of an A.beta. aggregation core domain and a modifying group coupled thereto such that the compound alters the aggregation or inhibits the neurotoxicity of natural .beta. amyloid peptides when contacted with the peptides. Furthermore, the modulators are capable of altering natural .beta.-AP aggregation when the natural .beta.-APs are in a molar excess amount relative to the modulators. Pharmaceutical compositions comprising the compounds of the invention, and diagnostic and treatment methods for amyloidogenic diseases using the compounds of the invention, are also disclosed.

Brief Summary Text (2):
Alzheimer's disease (AD), first described by the Bavarian psychiatrist Alois Alzheimer in 1907, is a progressive neurological disorder that begins with short term memory loss and proceeds to disorientation, impairment of judgment and reasoning and, ultimately, dementia. The course of the disease usually leads to death in a severely debilitated, immobile state between four and 12 years after onset. AD has been estimated to afflict 5 to 11 percent of the population over age 65 and as much as 47 percent of the population over age 85. The societal cost for managing AD is upwards of 80 billion dollars annually, primarily due to the extensive custodial care required for AD patients. Moreover, as adults born during the population boom of the 1940's and 1950's approach the age when AD becomes more prevalent, the control and treatment of AD will become an even more significant health care problem. Currently, there is no treatment that significantly retards the progression of the disease. For reviews on AD, see Selkoe, D. J. Sci. Amer., November 1991, pp. 68-78; and Yankner, B. A. et al. (1991) N. Eng. J. Med. 325:1849-1857.

Brief Summary Text (3):
It has recently been reported (Games et al. (1995) Nature 373:523-527) that an Alzheimer-type neuropathology has been created in transgenic mice. The transgenic mice express high levels of human mutant amyloid precursor protein and progressively develop many of the pathological conditions associated with AD.

Brief Summary Text (4):
Pathologically, AD is characterized by the presence of distinctive lesions in the victim's brain. These brain lesions include abnormal intracellular filaments called neurofibrillary tangles (NTFs) and extracellular deposits of amyloidogenic proteins in senile, or amyloid, plaques. Amyloid deposits are also present in the walls of cerebral blood vessels of AD patients. The major protein constituent of amyloid plaques has been identified as a 4 kilodalton peptide called .beta.-amyloid peptide (.beta.-AP)(Glenner, G. G. and Wong, C. W. (1984) Biochem. Biophys. Res. Commun. 120:885-890; Masters, C. et al. (1985) Proc. Natl. Acad. Sci. USA 82:4245-4249). Diffuse deposits of .beta.-AP are frequently observed in normal adult brains, whereas AD brain tissue is characterized by more compacted, dense-core .beta.-amyloid plaques. (See e.g., Davies, L. et al. (1988) Neurology 38:1688-1693). These observations suggest that .beta.-AP deposition precedes, and contributes to, the destruction of neurons that occurs in AD. In further support of a direct pathogenic role for .beta.-AP, .beta.-amyloid has been shown to be toxic to mature neurons, both in culture and in vivo. Yankner, B. A. et al. (1989) Science 245:417-420; Yankner, B. A. et al. (1990) Proc. Natl. Acad. Sci. USA 87:9020-9023;

Roher, A. E. et al. (1991) Biochem. Biophys. Res. Commun. 174:572-579; Kowall, N. W. et al. (1991) Proc. Natl. Acad. Sci. USA 88:7247-7251. Furthermore, patients with hereditary cerebral hemorrhage with amyloidosis-Dutch-type (HCHWA-D), which is characterized by diffuse .beta.-amyloid deposits within the cerebral cortex and cerebrovasculature, have been shown to have a point mutation that leads to an amino acid substitution within .beta.-AP. Levy, E. et al. (1990) Science 248:1124-1126. This observation demonstrates that a specific alteration of the .beta.-AP sequence can cause .beta.-amyloid to be deposited.

Brief Summary Text (5):
Natural .beta.-AP is derived by proteolysis from a much larger protein called the amyloid precursor protein (APP). Kang, J. et al. (1987) Nature 325:733; Goldgaber, D. et al. (1987) Science 235:877; Robakis, N. K. et al. (1987) Proc. Natl. Acad. Sci. USA 84:4190; Tanzi, R. E. et al. (1987) Science 235:880. The APP gene maps to chromosome 21, thereby providing an explanation for the .beta.-amyloid deposition seen at an early age in individuals with Down's syndrome, which is caused by trisomy of chromosome 21. Mann, D. M. et al. (1989) Neuropathol. Appl. Neurobiol. 15:317; Rumble, B. et al. (1989) N. Eng. J Med. 320:1446. APP contains a single membrane spanning domain, with a long amino terminal region (about two-thirds of the protein) extending into the extracellular environment and a shorter carboxy-terminal region projecting into the cytoplasm. Differential splicing of the APP messenger RNA leads to at least five forms of APP, composed of either 563 amino acids (APP-563), 695 amino acids (APP-695), 714 amino acids (APP-714), 751 amino acids (APP-751) or 770 amino acids (APP-770).

Brief Summary Text (6):
Within APP, naturally-occurring .beta. amyloid peptide begins at an aspartic acid residue at amino acid position 672 of APP-770. Naturally-occurring .beta.-AP derived from proteolysis of APP is 39 to 43 amino acid residues in length, depending on the carboxy-terminal end point, which exhibits heterogeneity. The predominant circulating form of .beta.-AP in the blood and cerebrospinal fluid of both AD patients and normal adults is .beta.1-40 ("short .beta."). Seubert, P. et al. (1992) Nature 359:325; Shoji, M. et al. (1992) Science 258:126. However, .beta.1-42 and .beta.1-43 ("long .beta.") also are forms in .beta.-amyloid plaques. Masters, C. et al. (1985) Proc. Natl Acad. Sci. USA 82:4245; Miller, D. et al. (1993) Arch. Biochem. Biophys. 301:41; Mori, H. et al. (1992) J. Biol. Chem. 267:17082. Although the precise molecular mechanism leading to .beta.-APP aggregation and deposition is unknown, the process has been likened to that of nucleation-dependent polymerizations, such as protein crystallization, microtubule formation and actin polymerization. See e.g., Jarrett, J. T. and Lansbury, P. T. (1993) Cell 73:1055-1058. In such processes, polymerization of monomer components does not occur until nucleus formation. Thus, these processes are characterized by a lag time before aggregation occurs, followed by rapid polymerization after nucleation. Nucleation can be accelerated by the addition of a "seed" or preformed nucleus, which results in rapid polymerization. The long .beta. forms of .beta.-AP have been shown to act as seeds, thereby accelerating polymerization of both long and short .beta.-AP forms. Jarrett, J. T. et al. (1993) Biochemistry 32:4693.

Brief Summary Text (7):
In one study, in which amino acid substitutions were made in .beta.-AP, two mutant .beta. peptides were reported to interfere with polymerization of non-mutated .beta.-AP when the mutant and non-mutant forms of peptide were mixed. Hilbich, C. et al. (1992) J. Mol. Biol. 228:460-473. However, equimolar amounts of the mutant and non-mutant (i.e., natural) .beta. amyloid peptides were used to see this effect and the mutant peptides were reported to be unsuitable for use in vivo. Hilbich, C. et al. (1992), supra.

Brief Summary Text (9):
This invention pertains to compounds, and pharmaceutical compositions thereof, that can modulate the aggregation of amyloidogenic proteins and peptides, in particular compounds that can modulate the aggregation of natural .beta. amyloid peptides (.beta.-AP) and inhibit the neurotoxicity of natural .beta.-APs. In one embodiment, the invention provides an amyloid modulator compound comprising an amyloidogenic protein, or peptide fragment thereof, coupled directly or indirectly to at least one modifying group such that the compound modulates the aggregation of natural amyloid proteins or peptides when contacted with the natural amyloidogenic proteins or peptides. Preferably, the compound inhibits aggregation of natural amyloidogenic proteins or peptides when contacted with the natural amyloidogenic proteins or peptides. The amyloidogenic protein, or peptide fragment thereof, can be, for example, selected from the group consisting of transthyretin (TTR), prion protein (PrP), islet amyloid polypeptide (IAPP), atrial natriuretic factor (ANF), kappa light chain, lambda light

chain, amyloid A, procalcitonin, cystatin C, .beta.2 microglobulin, ApoA-I, gelsolin, calcitonin, fibrinogen and lysozyme.

Brief Summary Text (10):
In the most preferred embodiment of the invention, the compound modulates the aggregation of natural .beta.-AP. The invention provides a .beta.-amyloid peptide compound comprising a formula: ##STR1## wherein Xaa is a .beta.-amyloid peptide having an amino-terminal amino acid residue corresponding to position 668 of .beta.-amyloid precursor protein-770 (APP-770) or to a residue carboxy-terminal to position 668 of APP-770, A is a modifying group attached directly or indirectly to the .beta.-amyloid peptide of the compound such that the compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides, and n is an integer selected such that the compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides.

Brief Summary Text (11):
In one embodiment, at least one A group is attached directly or indirectly to the amino terminus of the .beta.-amyloid peptide of the compound. In another embodiment, at least one A group is attached directly or indirectly to the carboxy terminus of the .beta.-amyloid peptide of the compound. In yet another embodiment, at least one A group is attached directly or indirectly to a side chain of at least one amino acid residue of the .beta.-amyloid peptide of the compound.

Brief Summary Text (12):
The invention also provides a .beta.-amyloid modulator compound comprising an A.beta. aggregation core domain (ACD) coupled directly or indirectly to at least one modifying group (MG) such that the compound modulates the aggregation or inhibits the neurotoxicity of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. Preferably, the A.beta. aggregation core domain is modeled after a subregion of natural .beta.-amyloid peptide between 3 and 10 amino acids in length.

Brief Summary Text (13):
The invention also provides .beta.-amyloid modulator compound comprising a formula: ##STR2## wherein Xaa.sub.1, Xaa.sub.2 and Xaa.sub.3 are each amino acid structures and at least two of Xaa.sub.1, Xaa.sub.2 and Xaa.sub.3 are, independently, selected from the group consisting of a leucine structure, a phenylalanine structure and a valine structure;

Brief Summary Text (14):
Y, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.a, wherein Xaa is any amino acid structure and a is an integer from 1 to 15;

Brief Summary Text (15):
Z, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.b, wherein Xaa is any amino acid structure and b is an integer from 1 to 15; and

Brief Summary Text (17):
Xaa.sub.1, Xaa.sub.2, Xaa.sub.3, Y, Z, A and n being selected such that the compound modulates the aggregation or inhibits the neurotoxicity of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. In a preferred embodiment, Xaa.sub.1 and Xaa.sub.2 are each phenylalanine structures. In another preferred embodiment Xaa.sub.2 and Xaa.sub.3 are each phenylalanine structures.

Brief Summary Text (18):
The invention further provides a .beta.-amyloid modulator compound comprising a formula: ##STR3## wherein Xaa.sub.1 and Xaa.sub.3 are amino acid structures; Xaa.sub.2 is a valine structure;

Brief Summary Text (20):
Y, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.a, wherein Xaa is any amino acid structure and a is an integer from 1 to 15;

Brief Summary Text (21):
Z, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.b, wherein Xaa is any amino acid structure and b is an integer from 1 to 15; and

Brief Summary Text (23):
Xaa.sub.1, Xaa.sub.3, Y, Z, A and n being selected such that the compound modulates the aggregation or inhibits the neurotoxicity of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. In a preferred embodiment, Xaa.sub.1 is a leucine structure and Xaa.sub.3 is phenylalanine structure.

Brief Summary Text (37):
The invention still further provides a .beta.-amyloid modulator compound comprising a modifying group attached directly or indirectly to a peptidic structure, wherein the peptidic structure comprises amino acid structures having an amino acid sequence selected from the group consisting of His-Gln-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:5), His-Gln-Lys-Leu-Val-Phe-Phe (SEQ ID NO:6), Gln-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:7), Gln-Lys-Leu-Val-Phe-Phe (SEQ ID NO:8), Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:9), Lys-Leu-Val-Phe-Phe (SEQ ID NO:10), Leu-Val-Phe-Phe-Ala (SEQ ID NO:11), Leu-Val-Phe-Phe (SEQ ID NO:12), Leu-Ala-Phe-Phe-Ala (SEQ ID NO:13), Val-Phe-Phe (SEQ ID NO:19), Phe-Phe-Ala (SEQ ID NO:20), Phe-Phe-Val-Leu-Ala (SEQ ID NO:21), Leu-Val-Phe-Phe-Lys (SEQ ID NO:22), Leu-Val-Iodotyrosine-Phe-Ala (SEQ ID NO:23), Val-Phe-Phe-Ala (SEQ ID NO:24), Ala-Val-Phe-Phe-Ala (SEQ ID NO:25), Leu-Val-Phe-Iodotyrosine-Ala (SEQ ID NO:26), Leu-Val-Phe-Phe-Ala-Glu (SEQ ID NO:27), Phe-Phe-Val-Leu (SEQ ID NO:28), Phe-Lys-Phe-Val-Leu (SEQ ID NO:29), Lys-Leu-Val-Ala-Phe (SEQ ID NO:30), Lys-Leu-Val-Phe-Phe-.beta.Ala (SEQ ID NO:31) and Leu-Val-Phe-Phe-DAla (SEQ ID NO:32).

Brief Summary Text (40):
The invention also provides a .beta.-amyloid modulator which inhibits aggregation of natural .beta.-amyloid peptides when contacted with a molar excess amount of natural .beta.-amyloid peptides.

Brief Summary Text (41):
The invention also provides a .beta.-amyloid peptide compound comprising an amino acid sequence having at least one amino acid deletion compared to .beta.AP.sub.1-39, such that the compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. In one embodiment, the compound has at least one internal amino acid deleted compared to .beta.AP.sub.1-39. In another embodiment, the compound has at least one N-terminal amino acid deleted compared to .beta.AP.sub.1-39. In yet another embodiment, the compound has at least one C-terminal amino acid deleted compared to .beta.AP.sub.1-39. Preferred compounds include .beta.AP.sub.6-20 (SEQ ID NO:4), .beta.AP.sub.16-30 (SEQ ID NO:14), .beta.AP.sub.1-20, 26-40 (SEQ ID NO:15) and EEVVHHHHQQ-.beta.AP.sub.16-40 (SEQ ID NO:16).

Brief Summary Text (42):
The compounds of the invention can be formulated into pharmaceutical compositions comprising the compound and a pharmaceutically acceptable carrier. The compounds can also be used in the manufacture of a medicament for the diagnosis or treatment of an amyloidogenic disease.

Brief Summary Text (43):
Another aspect of the invention pertains to diagnostic and treatment methods using the compounds of the invention. The invention provides a method for inhibiting aggregation of natural .beta.-amyloid peptides, comprising contacting the natural .beta.-amyloid peptides with a compound of the invention such that aggregation of the natural .beta.-amyloid peptides is inhibited. The invention also provides a method for inhibiting neurotoxicity of natural .beta.-amyloid peptides, comprising contacting the natural .beta.-amyloid peptides with a compound of the invention such that neurotoxicity of the natural .beta.-amyloid peptides is inhibited.

Brief Summary Text (44):
In another embodiment, the invention provides a method for detecting the presence or absence of natural .beta.-amyloid peptides in a biological sample, comprising contacting a biological sample with a compound of the invention and detecting the compound bound to natural .beta.-amyloid peptides to thereby detect the presence or absence of natural .beta.-amyloid peptides in the biological sample. In one embodiment, the .beta.-amyloid modulator compound and the biological sample are contacted in vitro. In another embodiment, the .beta.-amyloid modulator compound is contacted with the biological sample by administering the .beta.-amyloid modulator compound to a subject. For in vivo administration, preferably the compound is labeled with radioactive technetium or radioactive iodine.

Brief Summary Text (45):
In another embodiment, the invention provides a method for detecting natural

.beta.-amyloid peptides to facilitate diagnosis of a .beta.-amyloidogenic disease, comprising contacting a biological sample with a compound of the invention and detecting the compound bound to natural .beta.-amyloid peptides to facilitate diagnosis of a .beta.-amyloidogenic disease. In one embodiment, the .beta.-amyloid modulator compound and the biological sample are contacted in vitro. In another embodiment, the .beta.-amyloid modulator compound is contacted with the biological sample by administering the .beta.-amyloid modulator compound to a subject. For in vivo administration, preferably the compound is labeled with radioactive technetium or radioactive iodine. Preferably, the method facilitates diagnosis of Alzheimer's disease.

Brief Summary Text (46):
The invention also provides a method for treating a subject for a disorder associated with amyloidosis, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound of the invention such that the subject is treated for a disorder associated with amyloidosis. The method can be used to treat disorders is selected, for example, from the group consisting of familial amyloid polyneuropathy (Portuguese, Japanese and Swedish types), familial amyloid cardiomyopathy (Danish type), isolated cardiac amyloid, systemic senile amyloidosis, scrapie, bovine spongiform encephalopathy, Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker syndrome, adult onset diabetes, insulinoma, isolated atrial amyloidosis, idiopathic (primary) amyloidosis, myeloma or macroglobulinemia-associated amyloidosis, primary localized cutaneous nodular amyloidosis associated with Sjogren's syndrome, reactive (secondary) amyloidosis, familial Mediterranean Fever and familial amyloid nephropathy with urticaria and deafness (Muckle-Wells syndrome), hereditary cerebral hemorrhage with amyloidosis of Icelandic type, amyloidosis associated with long term hemodialysis, hereditary non-neuropathic systemic amyloidosis (familial amyloid polyneuropathy III), familial amyloidosis of Finnish type, amyloidosis associated with medullary carcinoma of the thyroid, fibrinogen-associated hereditary renal amyloidosis and lysozyme-associated hereditary systemic amyloidosis.

Brief Summary Text (47):
In a preferred embodiment, the invention provides a method for treating a subject for a disorder associated with .beta.-amyloidosis, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound of the invention such that the subject is treated for a disorder associated with .beta.-amyloidosis. Preferably the disorder is Alzheimer's disease.

Brief Summary Text (48):
In yet another embodiment, the invention provides a method for treating a subject for a disorder associated with .beta.-amyloidosis, comprising administering to the subject a recombinant expression vector encoding a peptide compound of the invention such that the compound is synthesized in the subject and the subject is treated for a disorder associated with .beta.-amyloidosis. Preferably, the disorder is Alzheimer's disease.

Drawing Description Text (2):
FIG. 1 is a graphic representation of the turbidity of a .beta.-AP.sub.1-40 solution, as measured by optical density at 400 nm, either in the absence of a .beta.-amyloid modulator or in the presence of the .beta.-amyloid modulator N-biotinyl-.beta.AP.sub.1-40 (1%, or 5%).

Drawing Description Text (3):
FIG. 2 is a schematic representation of compounds which can be used to modify a .beta.-AP or an A.beta. aggregation core domain to form a .beta.-amyloid modulator of the invention.

Detailed Description Text (2):
This invention pertains to compounds, and pharmaceutical compositions thereof, that can modulate the aggregation of amyloidogenic proteins and peptides, in particular compounds that can modulate the aggregation of natural (D amyloid) peptides (.beta.-AP) and inhibit the neurotoxicity of natural .beta.-APs. A compound of the invention that modulates aggregation of natural .beta.-AP, referred to herein interchangeably as a .beta. amyloid modulator compound, a .beta. amyloid modulator or simply a modulator, alters the aggregation of natural .beta.-AP when the modulator is contacted with natural .beta.-AP. Thus, a compound of the invention acts to alter the natural aggregation process or rate for .beta.-AP, thereby disrupting this process. Preferably, the compounds inhibit .beta.-AP aggregation. Furthermore, the invention provides subregions of the .beta. amyloid peptide that are sufficient, when appropriately

modified as described herein, to alter (and preferably inhibit) aggregation of natural .beta. amyloid peptides when contacted with the natural .beta. amyloid peptides. In particular, preferred modulator compounds of the invention are comprised of a modified form of an A.beta. aggregation core domain, modeled after the aforementioned A.beta. subregion (as described further below), which is sufficient to alter (and preferably inhibit) the natural aggregation process or rate for .beta.-AP. This A.beta. aggregation core domain can comprises as few as three amino acid residues (or derivative, analogues or mimetics thereof). Moreover, while the amino acid sequence of the A.beta. aggregation core domain can directly correspond to an amino acid sequence found in natural .beta.-AP, it is not essential that the amino acid sequence directly correspond to a .beta.-AP sequence. Rather, amino acid residues derived from a preferred subregion of .beta.-AP (a hydrophobic region centered around positions 17-20) can be rearranged in order and/or substituted with homologous residues within a modulator compound of the invention and yet maintain their inhibitory activity (described further below).

Detailed Description Text (3):
The .beta. amyloid modulator compounds of the invention can be selected based upon their ability to inhibit the aggregation of natural .beta.-AP in vitro and/or inhibit the neurotoxicity of natural .beta.-AP fibrils for cultured cells (using assays described herein). Accordingly, the preferred modulator compounds inhibit the aggregation of natural .beta.-AP and/or inhibit the neurotoxicity of natural .beta.-AP. However, modulator compounds selected based on one or both of these properties may have additional properties in vivo that may be beneficial in the treatment of amyloidosis. For example, the modulator compound may interfere with processing of natural .beta.-AP (either by direct or indirect protease inhibition) or by modulation of processes that produce toxic .beta.-AP, or other APP fragments, in vivo. Alternatively, modulator compounds may be selected based on these latter properties, rather than inhibition of A.beta. aggregation in vitro. Moreover, modulator compounds of the invention that are selected based upon their interaction with natural .beta.-AP also may interact with APP or with other APP fragments.

Detailed Description Text (4):
As used herein, a "modulator" of .beta.-amyloid aggregation is intended to refer to an agent that, when contacted with natural .beta. amyloid peptides, alters the aggregation of the natural .beta. amyloid peptides. The term "aggregation of .beta. amyloid peptides" refers to a process whereby the peptides associate with each other to form a multimeric, largely insoluble complex. The term "aggregation" further is intended to encompass .beta. amyloid fibril formation and also encompasses .beta.-amyloid plaques.

Detailed Description Text (5):
The terms "natural .beta.-amyloid peptide", "natural .beta.-AP" and "natural A.beta. peptide", used interchangeably herein, are intended to encompass naturally occurring proteolytic cleavage products of the .beta. amyloid precursor protein (APP) which are involved in .beta.-AP aggregation and .beta.-amyloidosis. These natural peptides include .beta.-amyloid peptides having 39-43 amino acids (i.e., A.beta..sub.1-39, A.beta..sub.1-40, A.beta..sub.1-41, A.beta..sub.1-42 and A.beta..sub.1-43). The amino-terminal amino acid residue of natural .beta.-AP corresponds to the aspartic acid residue at position 672 of the 770 amino acid residue form of the amyloid precursor protein ("APP-770"). The 43 amino acid long form of natural .beta.-AP has the amino acid sequence.

Detailed Description Text (7):
(also shown in SEQ ID NO:1), whereas the shorter forms have 1-4 amino acid residues deleted from the carboxy-terminal end. The amino acid sequence of APP-770 from position 672 (i.e., the amino-terminus of natural .beta.-AP) to its C-terminal end (103 amino acids) is shown in SEQ ID NO:2. The preferred form of natural .beta.-AP for use in the aggregation assays described herein is A.beta..sub.1-40.

Detailed Description Text (8):
In the presence of a modulator of the invention, aggregation of natural .beta. amyloid peptides is "altered" or "modulated". The various forms of the term "alteration" or "modulation" are intended to encompass both inhibition of .beta.-AP aggregation and promotion of .beta.-AP aggregation. Aggregation of natural .beta.-AP is "inhibited" in the presence of the modulator when there is a decrease in the amount and/or rate of .beta.-AP aggregation as compared to the amount and/or rate of .beta.-AP aggregation in the absence of the modulator. The various forms of the term "inhibition" are intended to include both complete and partial inhibition of .beta.-AP aggregation. Inhibition of aggregation can be quantitated as the fold increase in the lag time for aggregation or

as the decrease in the overall plateau level of aggregation (i.e., total amount of aggregation), using an aggregation assay as described in the Examples. In various embodiments, a modulator of the invention increases the lag time of aggregation at least 1.2-fold, 1.5-fold, 1.8-fold, 2-fold, 2.5-fold, 3-fold, 4-fold or 5-fold. In various other embodiments, a modulator of the invention inhibits the plateau level of aggregation at least 10%, 20%, 30%, 40%, 50%, 75% or 100%.

Detailed Description Text (9):
A modulator which inhibits .beta.-AP aggregation (an "inhibitory modulator compound") can be used to prevent or delay the onset of .beta.-amyloid deposition. Moreover, as demonstrated in Example 10, inhibitory modulator compounds of the invention inhibit the formation and/or activity of neurotoxic aggregates of natural A.beta. peptide (i.e., the inhibitory compounds can be used to inhibit the neurotoxicity of .beta.-AP). Still further, also as demonstrated in Example 10, the inhibitory compounds of the invention can be used to reduce the neurotoxicity of preformed .beta.-AP aggregates, indicating that the inhibitory modulators can either bind to preformed A.beta. fibrils or soluble aggregate and modulate their inherent neurotoxicity or that the modulators can perturb the equilibrium between monomeric and aggregated forms of .beta.-AP in favor of the non-neurotoxic form.

Detailed Description Text (10):
Alternatively, in another embodiment, a modulator compound of the invention promotes the aggregation of natural A.beta. peptides. The various forms of the term "promotion" refer to an increase in the amount and/or rate of .beta.-AP aggregation in the presence of the modulator, as compared to the amount and/or rate of .beta.-AP aggregation in the absence of the modulator. Such a compound which promotes A.beta. aggregation is referred to as a stimulatory modulator compound. Stimulatory modulator compounds may be useful for sequestering .beta.-amyloid peptides, for example in a biological compartment where aggregation of .beta.-AP may not be deleterious to thereby deplete .beta.-AP from a biological compartment where aggregation of .beta.-AP is deleterious. Moreover, stimulatory modulator compounds can be used to promote A.beta. aggregation in in vitro aggregation assays (e.g., assays such as those described in the Examples), for example in screening assays for test compounds that can then inhibit or reverse this A.beta. aggregation (i.e., a stimulatory modulator compound can act as a "seed" to promote the formation of A.beta. aggregates).

Detailed Description Text (11):
In a preferred embodiment, the modulators of the invention are capable of altering .beta.-AP aggregation when contacted with a molar excess amount of natural .beta.-AP. A "molar excess amount of natural .beta.-AP" refers to a concentration of natural .beta.-AP, in moles, that is greater than the concentration, in moles, of the modulator. For example, if the modulator and .beta.-AP are both present at a concentration of 1 .mu.M, they are said to be "equimolar", whereas if the modulator is present at a concentration of 1 .mu.M and the .beta.-AP is present at a concentration of 5 .mu.M, the .beta.-AP is said to be present at a 5-fold molar excess amount compared to the modulator. In preferred embodiments, a modulator of the invention is effective at altering natural .beta.-AP aggregation when the natural .beta.-AP is present at at least a 2-fold, 3-fold or 5-fold molar excess compared to the concentration of the modulator. In other embodiments, the modulator is effective at altering .beta.-AP aggregation when the natural .beta.-AP is present at at least a 10-fold, 20-fold, 33-fold, 50-fold, 100-fold, 500-fold or 1000-fold molar excess compared to the concentration of the modulator.

Detailed Description Text (14):
In one embodiment, a modulator of the invention comprises a .beta.-amyloid peptide compound comprising the formula: ##STR4## wherein Xaa is a .beta.-amyloid peptide, A is a modulating group attached directly or indirectly to the .beta.-amyloid peptide of the compound such that the compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides, and n is an integer selected such that the compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides.

Detailed Description Text (15):
Preferably, .beta.-amyloid peptide of the compound has an amino-terminal amino acid residue corresponding to position 668 of .beta.-amyloid precursor protein-770 (APP-770) or to a residue carboxy-terminal to position 668 of APP-770. The amino acid sequence of APP-770 from position 668 to position 770 (i.e., the carboxy terminus) is shown below and in SEQ ID NO:2:

Detailed Description Text (17):
More preferably, the amino-terminal amino acid residue of the .beta.-amyloid peptide corresponds to position 672 of APP-770 (position 5 of the amino acid sequence of SEQ ID NO:2) or to a residue carboxy-terminal to position 672 of APP-770. Although the .beta.-amyloid peptide of the compound may encompass the 103 amino acid residues corresponding to positions 668-770 of APP-770, preferably the peptide is between 6 and 60 amino acids in length, more preferably between 10 and 43 amino acids in length and even more preferably between 10 and 25 amino acid residues in length.

Detailed Description Text (18):
As used herein, the term ".beta. amyloid peptide", as used in a modulator of the invention is intended to encompass peptides having an amino acid sequence identical to that of the natural sequence in APP, as well as peptides having acceptable amino acid substitutions from the natural sequence. Acceptable amino acid substitutions are those that do not affect the ability of the peptide to alter natural .beta.-AP aggregation. Moreover, particular amino acid substitutions may further contribute to the ability of the peptide to alter natural .beta.-AP aggregation and/or may confer additional beneficial properties on the peptide (e.g., increased solubility, reduced association with other amyloid proteins, etc.). For example, substitution of hydrophobic amino acid residues for the two phenylalanine residues at positions 19 and 20 of natural .beta.-AP (positions 19 and 20 of the amino acid sequence shown in SEQ ID NO:1) may further contribute to the ability of the peptide to alter .beta.-AP aggregation (see Hilbich, C. (1992) J. Mol. Biol. 228:460-473). Thus, in one embodiment, the .beta.-AP of the compound consists of the amino acid sequence shown below and in SEQ ID NO:3:

Detailed Description Text (20):
(or an amino-terminal or carboxy-terminal deletion thereof), wherein Xaa is a hydrophobic amino acid. Examples of hydrophobic amino acids are isoleucine, leucine, threonine, serine, alanine, valine or glycine. Preferably, F.sub.19 F.sub.20 is substituted with T.sub.19 T.sub.20 or G.sub.19 I.sub.20.

Detailed Description Text (21):
Other suitable amino acid substitutions include replacement of amino acids in the human peptide with the corresponding amino acids of the rodent .beta.-AP peptide. The three amino acid residues that differ between human and rat .beta.-AP are at positions 5, 10 and 13 of the amino acid sequence shown in SEQ ID NOs:1 and 3. A human .beta.-AP having the human to rodent substitutions Arg.sub.5 to Gly, Tyr.sub.10 to Phe and His.sub.13 to Arg has been shown to retain the properties of the human peptide (see Fraser, P. E. et al. (1992) Biochemistry 31:10716-10723; and Hilbich, C. et al. (1991) Eur. J. Biochem. 201:61-69). Accordingly, a human .beta.-AP having rodent .beta.-AP a.a. substitutions is suitable for use in a modulator of the invention.

Detailed Description Text (22):
Other possible .beta.-AP amino acid substitutions are described in Hilbich, C. et al. (1991) J. Mol. Biol. 218:149-163; and Hilbich, C. (1992) J. Mol. Biol. 228:460-473. Moreover, amino acid substitutions that affect the ability of .beta.-AP to associate with other proteins can be introduced. For example, one or more amino acid substitutions that reduce the ability of .beta.-AP to associate with the serpin enzyme complex (SEC) receptor, .alpha.1-antichymotrypsin (ACT) and/or apolipoprotein E (ApoE) can be introduced. A preferred substitution for reducing binding to the SEC receptor is L.sub.34 M.sub.35 to A.sub.34 A.sub.35 (at positions 34 and 35 of the amino acid sequences shown in SEQ ID NOs:1 and 3). A preferred substitution for reducing binding to ACT is S.sub.8 to A.sub.8 (at position 8 of the amino acid sequences shown in SEQ ID NOs:1 and 3).

Detailed Description Text (23):
Alternative to .beta.-AP amino acid substitutions described herein or known in the art, a modulator composed, at least in part, of an amino acid-substituted .beta. amyloid peptide can be prepared by standard techniques and tested for the ability to alter .beta.-AP aggregation using an aggregation assay described herein. To retain the properties of the original modulator, preferably conservative amino acid substitutions are made at one or more amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g. glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), .beta.-branched side chains (e.g., threonine,

valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Accordingly, a modulator composed of a .beta. amyloid peptide having an amino acid sequence that is mutated from that of the wild-type sequence in APP-770 yet which still retains the ability to alter natural .beta.-AP aggregation is within the scope of the invention.

Detailed Description Text (24):
As used herein, the term ".beta. amyloid peptide" is further intended to include peptide analogues or peptide derivatives or peptidomimetics that retain the ability to alter natural .beta.-AP aggregation as described herein. For example, a .beta. amyloid peptide of a modulator of the invention may be modified to increase its stability, bioavailability, solubility, etc. The terms "peptide analogue", "peptide derivative" and "peptidomimetic" as sued herein are intended to include molecules which mimic the chemical structure of a peptide and retain the functional properties of the peptide. Approaches to designing peptide analogs are known in the art. For example, see Farmer, P. S. in Drug Design (E. J. Ariens, ed.) Academic Press, New York, 1980, vol. 10, pp. 119-143; Ball. J. B. and Alewood, P. F. (1990) J. Mol. Recognition 3:55; Morgan, B. A. and Gainor, J. A. (1989) Ann. Rep. Med. Chem. 24:243; and Freidinger, R. M. (1989) Trends Pharmacol. Sci. 10:270. Examples of peptide analogues, derivatives and peptidomimetics include peptides substituted with one or more benzodiazepine molecules (see e.g., James, G. L. et al. (1993) Science 260:1937-1942), peptides with methylated amide linkages and "retro-inverso" peptides (see U.S. Pat. No. 4,522,752 by Sisto). Peptide analogues, peptide derivatives and peptidomimetic are described in further detail below with regard to compounds comprising an A.beta. aggregation core domain.

Detailed Description Text (25):
In a modulator of the invention having the formula shown above, a modulating group ("A") is attached directly or indirectly to the .beta.-amyloid peptide of the modulator (As used herein, the term "modulating group" and "modifying group" are used interchangeably to describe a chemical group directly or indirectly attached to an A.beta. derived peptidic structure). For example, the modulating group can be directly attached by covalent coupling to the .beta.-amyloid peptide or the modulating group can be attached indirectly by a stable non-covalent association. In one embodiment of the invention, the modulating group is attached to the amino-terminus of the .beta.-amyloid peptide of the modulator. Accordingly, the modulator can comprise a compound having a formula: ##STR5##

Detailed Description Text (26):
Alternatively, in another embodiment of the invention, the modulating group is attached to the carboxy-terminus of the .beta.-amyloid peptide of the modulator. Accordingly, the modulator can comprise a compound having a formula: ##STR6##

Detailed Description Text (27):
In yet another embodiment, the modulating group is attached to the side chain of at least one amino acid residue of the .beta.-amyloid peptide of the compound (e.g., through the epsilon amino group of a lysyl residue(s), through the carboxyl group of an aspartic acid residue(s) or a glutamic acid residue(s), through a hydroxy group of a tyrosyl residue(s), a serine residue(s) or a threonine residue(s) or other suitable reactive group on an amino acid side chain).

Detailed Description Text (28):
The modulating group is selected such that the compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. Accordingly, since the .beta.-AP peptide of the compound is modified from its natural state, the modulating group "A" as used herein is not intended to include hydrogen. In a preferred embodiment, the modulating group is a biotin compound of the formula: ##STR7## wherein X.sub.1 -X.sub.3 are each independently selected from the group consisting of S, O and NR.sub.2, wherein R.sub.2 is hydrogen, or an aryl, lower alkyl, alkenyl or alkynyl moiety; W is O or NR.sub.2 ; R.sub.1 is a lower alkylenyl moiety and Y is a direct bond or a spacer molecule selected for its ability to react with a target group on a .beta.-AP. At least one of X.sub.1 -X.sub.3 is an NR.sub.2 group or W is an N(R.sub.2).sub.2 group.

Detailed Description Text (31):
The spacer molecule (Y) can be, for example, a lower alkyl group or a linker peptide, and is preferably selected for its ability to link with a free amino group (e.g., the .alpha.-amino group at the amino-terminus of a .beta.-AP). Thus, in a preferred embodiment, the biotin compound modifies the amino-terminus of a .beta.-amyloid peptide.

Detailed Description Text (32):
Additional suitable modulating groups may include other cyclic and heterocyclic compounds and other compounds having similar steric "bulk". Non-limiting examples of compounds which can be used to modify a .beta.-AP are shown schematically in FIG. 2, and include N-acetylneuraminic acid, cholic acid, trans-4-cotininecarboxylic acid, 2-imino-1-imidazolidineacetic acid, (S)-(-)-indoline-2-carboxylic acid, (-)-menthoxyacetic acid, 2-norbornaneacetic acid, .gamma.-oxo-5-acenaphthenebutyric acid, (-)-2-oxo-4-thiazolidinecarboxylic acid, tetrahydro-3-furoic acid, 2-iminobiotin-N-hydroxysuccinimide ester, diethylenetriaminepentaacetic dianhydride, 4-morpholinecarbonyl chloride, 2-thiopheneacetyl chloride, 2-thiophenesulfonyl chloride, 5-(and 6-)-carboxyfluorescein (succinimidyl ester), fluorescein isothiocyanate, and acetic acid (or derivatives thereof). Suitable modulating groups are described further in subsection II below.

Detailed Description Text (33):
In a modulator of the invention, a single modulating group may be attached to a .beta.-amyloid peptide (e.g., n=1 in the formula shown above) or multiple modulating groups may be attached to the peptide. The number of modulating groups is selected such that the compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. However, n preferably is an integer between 1 and 60, more preferably between 1 and 30 and even more preferably between 1 and 10 or 1 and 5.

Detailed Description Text (34):
In another embodiment, a .beta.-amyloid modulator compound of the invention comprises an A.beta. aggregation core domain (abbreviated as ACD) coupled directly or indirectly to a modifying group such that the compound modulates the aggregation or inhibits the neurotoxicity of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. As used herein, an "A.beta. aggregation core domain" is intended to refer to a structure that is modeled after a subregion of a natural .beta.-amyloid peptide which is sufficient to modulate aggregation of natural .beta.-APs when this subregion of the natural .beta.-AP is appropriately modified as described herein (e.g., modified at the amino-terminus). The term "subregion of a natural .beta.-amyloid peptide" is intended to include amino-terminal and/or carboxy-terminal deletions of natural .beta.-AP. The term "subregion of natural .beta.-AP" is not intended to include full-length natural .beta.-AP (i.e., "subregion" does not include A.beta..sub.1-39, A.beta..sub.1-40, A.beta..sub.1-41, A.beta..sub.1-42 and A.beta..sub.1-43).

Detailed Description Text (35):
Although not intending to be limited by mechanism, the ACD of the modulators of the invention is thought to confer a specific targeting function on the compound that allows the compound to recognize and specifically interact with natural .beta.-AP. Preferably, the ACD is modeled after a subregion of natural .beta.-AP that is less than 15 amino acids in length and more preferably is between 3-10 amino acids in length. In various embodiments, the ACD is modeled after a subregion of .beta.-AP that is 10, 9, 8, 7, 6, 5, 4 or 3 amino acids in length. In one embodiment, the subregion of .beta.-AP upon which the ACD is modeled is an internal or carboxy-terminal region of .beta.-AP (i.e., downstream of the amino-terminus at amino acid position 1). In another embodiment, the ACD is modeled after a subregion of .beta.-AP that is hydrophobic. In certain specific embodiments, the term A.beta. aggregation core domain specifically excludes .beta.-AP subregions corresponding to amino acid positions 1-15 (A.beta..sub.1-15), 6-20 (A.beta..sub.6-20) and 16-40 (A.beta..sub.16-40).

Detailed Description Text (36):
An A.beta. aggregation core domain can be comprised of amino acid residues linked by peptide bonds. That is, the ACD can be a peptide corresponding to a subregion of .beta.-AP. Alternatively, an A.beta. aggregation core domain can be modeled after the natural A.beta. peptide region but may be comprised of a peptide analogue, peptide derivative or peptidomimetic compound, or other similar compounds which mimics the structure and function of the natural peptide. Accordingly, as used herein, an "A.beta. aggregation core domain" is intended to include peptides, peptide analogues, peptide derivatives and peptidomimetic compounds which, when appropriately modified, retain the aggregation modulatory activity of the modified natural A.beta. peptide subregion. Such structures that are designed based upon the amino acid sequence are referred to herein as "A.beta. derived peptidic structures." Approaches to designing peptide analogues, derivatives and mimetics are known in the art. For example, see Farmer, P. S. in Drug Design (E. J. Ariens, ed.) Academic Press, New York, 1980, vol. 10, pp. 119-143; Ball.

J. B. and Alewood, P. F. (1990) J. Mol. Recognition 3:55; Morgan, B. A. and Gainor, J. A. (1989) Ann. Rep. Med Chem. 24:243; and Freidinger, R. M. (1989) Trends Pharmacol. Sci. 10:270. See also Sawyer, T. K. (1995) "Peptidomimetic Design and Chemical Approaches to Peptide Metabolism" in Taylor, M. D. and Amidon, G. L. (eds.) Peptide-Based Drug Design: Controlling Transport and Metabolism, Chapter 17; Smith, A. B. 3rd, et al. (1995) J. Am. Chem. Soc. 117:11113-11123; Smith, A. B. 3rd, et al. (1994) J. Am. Chem. Soc. 116:9947-9962; and Hirschman, R., et al. (1993) J. Am. Chem. Soc. 115:12550-12568.

Detailed Description Text (37):
As used herein, a "derivative" of a compound X (e.g., a peptide or amino acid) refers to a form of X in which one or more reaction groups on the compound have been derivatized with a substituent group. Examples of peptide derivatives include peptides in which an amino acid side chain, the peptide backbone, or the amino- or carboxy-terminus has been derivatized (e.g., peptidic compounds with methylated amide linkages). As used herein an "analogue" of a compound X refers to a compound which retains chemical structures of X necessary for functional activity of X yet which also contains certain chemical structures which differ from X. An examples of an analogue of a naturally-occurring peptide is a peptides which includes one or more non-naturally-occurring amino acids. As used herein, a "mimetic" of a compound X refers to a compound in which chemical structures of X necessary for functional activity of X have been replaced with other chemical structures which mimic the conformation of X. Examples of peptidomimetics include peptidic compounds in which the peptide backbone is substituted with one or more benzodiazepine molecules (see e.g., James, G. L. et al. (1993) Science 260:1937-1942), peptides in which all L-amino acids are substituted with the corresponding D-amino acids and "retro-inverso" peptides (see U.S. Pat. No. 4,522,752 by Sisto), described further below.

Detailed Description Text (39):
Other possible modifications include an N-alkyl (or aryl) substitution (.psi.[CONR]), backbone crosslinking to construct lactams and other cyclic structures, substitution of all D-amino acids for all L-amino acids within the compound ("inverso" compounds) or retro-inverso amino acid incorporation (.psi.[NHCO]). By "inverso" is meant replacing L-amino acids of a sequence with D-amino acids, and by "retro-inverso" or "enantio-retro" is meant reversing the sequence of the amino acids ("retro") and replacing the L-amino acids with D-amino acids. For example, if the parent peptide is Thr-Ala-Tyr, the retro modified form is Tyr-Ala-Thr, the inverso form is thr-ala-tyr, and the retro-inverso form is tyr-ala-thr (lower case letters refer to D-amino acids). Compared to the parent peptide, a retro-inverso peptide has a reversed backbone while retaining substantially the original spatial conformation of the side chains, resulting in a retro-inverso isomer with a topology that closely resembles the parent peptide. See Goodman et al. "Perspectives in Peptide Chemistry" pp. 283-294 (1981). See also U.S. Pat. No. 4,522,752 by Sisto for further description of "retro-inverso" peptides.

Detailed Description Text (41):
In a preferred embodiment, the ACD of the modulator is modeled after the subregion of .beta.-AP encompassing amino acid positions 17-20 (i.e., Leu-Val-Phe-Phe; SEQ ID NO:12). As described further in Examples 7, 8 and 9, peptide subregions of A.beta..sub.1-40 were prepared, amino-terminally modified and evaluated for their ability to modulate aggregation of natural .beta.-amyloid peptides. One subregion that was effective at inhibiting aggregation was A.beta..sub.6-20 (i.e., amino acid residues 6-20 of the natural A.beta..sub.1-40 peptide, the amino acid sequence of which is shown in SEQ ID NO:4). Amino acid residues were serially deleted from the amino-terminus or carboxy terminus of this subregion to further delineate a minimal subregion that was sufficient for aggregation inhibitory activity. This process defined A.beta..sub.17-20 (i.e., amino acid residues 17-20 of the natural A.beta..sub.1-40 peptide) as a minimal subregion that, when appropriately modified, is sufficient for aggregation inhibitory activity. Accordingly, an "A.beta. aggregation core domain" within a modulator compound of the invention can be modeled after A.beta..sub.17-20. In one embodiment, the A.beta. aggregation core domain comprises A.beta..sub.17-20 itself (i.e., a peptide comprising the amino acid sequence leucine-valine-phenylalanine-phenylalanine; SEQ ID NO:12). In other embodiments, the structure of A.beta..sub.17-20 is used as a model to design an A.beta. aggregation core domain having similar structure and function to A.beta..sub.17-20. For example, peptidomimetics, derivatives or analogues of A.beta..sub.17-20 (as described above) can be used as an A.beta. aggregation core domain. In addition to A.beta..sub.17-20, the natural A.beta. peptide is likely to contain other minimal subregions that are sufficient for aggregation inhibitory activity. Such additional minimal subregions can be identified by the processes described in Examples 7, 8 and 9, wherein a 15 mer subregion of A.beta..sub.1-40 is

serially deleted from the amino-terminus or carboxy terminus, the deleted peptides are appropriately modified and then evaluated for aggregation inhibitory activity.

Detailed Description Text (42):
One form of the .beta.-amyloid modulator compound comprising an A.beta. aggregation core domain modeled after A.beta..sub.17-20 coupled directly or indirectly to at least one modifying group has the formula: ##STR8## wherein Xaa.sub.1 and Xaa.sub.3 are amino acid structures;

Detailed Description Text (45):
Y, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.a, wherein Xaa is any amino acid structure and a is an integer from 1 to 15;

Detailed Description Text (46):
Z, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.b, wherein Xaa is any amino acid structure and b is an integer from 1 to 15; and

Detailed Description Text (48):
Xaa.sub.1, Xaa.sub.3, Y, Z, A and n being selected such that the compound modulates the aggregation or inhibits the neurotoxicity of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides.

Detailed Description Text (49):
Preferably, a modulator compound of the above formula inhibits aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides and/or inhibits A.beta. neurotoxicity. Alternatively, the modulator compound can promote aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. The type and number of modifying groups ("A") coupled to the modulator are selected such that the compound alters (and preferably inhibits) aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. A single modifying group can be coupled to the modulator (i.e., n=1 in the above formula) or, alternatively, multiple modifying groups can be coupled to the modulator. In various embodiments, n is an integer between 1 and 60, between 1 and 30, between 1 and 10, between 1 and 5 or between 1 and 3. Suitable types of modifying groups are described further in subsection II below.

Detailed Description Text (50):
As demonstrated in Example 9, amino acid positions 18 (Val.sub.18) and 20 (Phe.sub.20) of A.beta..sub.17-20 (corresponding to Xaa.sub.2 and Xaa.sub.4) are particularly important within the core domain for inhibitory activity of the modulator compound. Accordingly, these positions are conserved within the core domain in the formula shown above. The terms "valine structure" and "phenylalanine structure" as used in the above formula are intended to include the natural amino acids, as well as non-naturally-occurring analogues, derivatives and mimetics of valine and phenylalanine, respectively, (including D-amino acids) which maintain the functional activity of the compound. Moreover, although Val.sub.18 and Phe.sub.20 have an important functional role, it is possible that Xaa.sub.2 and/or Xaa.sub.4 can be substituted with other naturally-occurring amino acids that are structurally related to valine or phenylalanine, respectively, while still maintaining the activity of the compound. Thus, the terms "valine structure" is intended to include conservative amino acid substitutions that retain the activity of valine at Xaa.sub.2, and the term "phenylalanine structure" is intended to include conservative amino acid substitutions that retain the activity of phenylalanine at Xaa.sub.4. However, the term "valine structure" is not intended to include threonine.

Detailed Description Text (51):
In contrast to positions 18 and 20 of A.beta..sub.17-20, a Phe to Ala substitution at position 19 (corresponding to Xaa.sub.3) did not abolish the activity of the modulator, indicating position 19 may be more amenable to amino acid substitution. In various embodiments of the above formula, positions Xaa.sub.1 and Xaa.sub.3 are any amino acid structure. The term "amino acid structure" is intended to include natural and non-natural amino acids as well as analogues, derivatives and mimetics thereof, including D-amino acids. In a preferred embodiment of the above formula, Xaa.sub.1 is a leucine structure and Xaa.sub.3 is a phenylalanine structure (i.e., modeled after Leu.sub.17 and Phe.sub.19, respectively, in the natural A.beta. peptide sequence). The term "leucine structure" is used in the same manner as valine structure and phenylalanine structure described above. Alternatively, an another embodiment, Xaa.sub.3 is an alanine structure.

Detailed Description Text (52):
The four amino acid structure ACD of the modulator of the above formula can be flanked at the amino-terminal side, carboxy-terminal side, or both, by peptidic structures derived either from the natural A.beta. peptide sequence or from non-A.beta. sequences. The term "peptidic structure" is intended to include peptide analogues, derivatives and mimetics thereof, as described above. The peptidic structure is composed of one or more linked amino acid structures, the type and number of which in the above formula are variable. For example, in one embodiment, no additional amino acid structures flank the Xaa.sub.1 -Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 core sequence (i.e., Y and Z are absent in the above formula). In another embodiment, one or more additional amino acid structures flank only the amino-terminus of the core sequences (i.e., Y is present but Z is absent in the above formula). In yet another embodiment, one or more additional amino acid structures flank only the carboxy-terminus of the core sequences (i.e., Z is present but Y is absent in the above formula). The length of flanking Z or Y sequences also is variable. For example, in one embodiment, a and b are integers from 1 to 15. More preferably, a and b are integers between 1 and 10. Even more preferably, a and b are integers between 1 and 5. Most preferably, a and b are integers between 1 and 3.

Detailed Description Text (53):
One form of the .beta.-amyloid modulator compound comprising an A.beta. aggregation core domain modeled after A.beta..sub.17-20 coupled directly or indirectly to at least one modifying group has the formula:

Detailed Description Text (55):
Xaa.sub.1 and Xaa.sub.3 are amino acids or amino acid mimetics;

Detailed Description Text (58):
Y, which may or may not be present, is a peptide or peptidomimetic having the formula (Xaa).sub.a, wherein Xaa is any amino acid or amino acid mimetic and a is an integer from 1 to 15;

Detailed Description Text (59):
Z, which may or may not be present, is a peptide or peptidomimetic having the formula (Xaa).sub.b, wherein Xaa is any amino acid or amino acid mimetic and b is an integer from 1 to 15; and

Detailed Description Text (61):
Xaa.sub.1, Xaa.sub.3, Y, Z, A and B being selected such that the compound modulates the aggregation or inhibits the neurotoxicity of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides.

Detailed Description Text (62):
In this embodiment, the modulator compound is specifically modified at either its amino-terminus, its carboxy-terminus, or both. The terminology used in this formula is the same as described above. Suitable modifying groups are described in subsection II below. In one embodiment, the compound is modified only at its amino terminus (i.e., B is absent and the compound comprises the formula: A-(Y)-Xaa.sub.1,-Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 -(Z)). In another embodiment, the compound is modified only at its carboxy-terminus (i.e., A is absent and the compound comprises the formula: (Y)-Xaa.sub.1 -Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 -(Z)-B). In yet another embodiment, the compound is modified at both its amino- and carboxy termini (i.e., the compound comprises the formula: A-(Y)-Xaa.sub.1 -Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 -(Z)-B and both A and B are present). As described above, the type and number of amino acid structures which flank the Xaa.sub.1 -Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 core sequences in the above formula is variable. For example, in one embodiment, a and b are integers from 1 to 15. More preferably, a and b are integers between 1 and 10. Even more preferably, a and b are integers between 1 and 5. Most preferably, a and b are integers between 1 and 3.

Detailed Description Text (77):
In one specific embodiment, the compound comprises the formula: A-Xaa.sub.4 -Xaa.sub.5 -Xaa.sub.6 -Xaa.sub.7 -B (e.g, a modified form of A.beta..sub.17-20, comprising an amino acid sequence Leu-Val-Phe-Phe; SEQ ID NO:12).

Detailed Description Text (78):
In another specific embodiment, the compound comprises the formula: A-Xaa.sub.4 -Xaa.sub.5 -Xaa.sub.6 -Xaa.sub.7 -Xaa.sub.8 -B (e.g, a modified form of A.beta..sub.17-21, comprising an amino acid sequence Leu-Val-Phe-Phe-Ala; SEQ ID NO:11).

Detailed Description Text (79):
In another specific embodiment, the compound comprises the formula: A-Xaa.sub.3 -Xaa.sub.4 -Xaa.sub.5 -Xaa.sub.6 -Xaa.sub.7 -B (e.g., a modified form of A.beta..sub.16-20, comprising an amino acid sequence Lys-Leu-Val-Phe-Phe; SEQ ID NO:10).

Detailed Description Text (80):
In another specific embodiment, the compound comprises the formula: A-Xaa.sub.3 -Xaa.sub.4 -Xaa.sub.5 -Xaa.sub.6 -Xaa.sub.7 -Xaa.sub.8 -B (e.g., a modified form of A.beta..sub.16-21, comprising an amino acid sequence Lys-Leu-Val-Phe-Phe-Ala; SEQ ID NO:9).

Detailed Description Text (81):
In another specific embodiment, the compound comprises the formula: A-Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 -Xaa.sub.5 -Xaa.sub.6 -Xaa.sub.7 -B (e.g., a modified form of A.beta..sub.15-20, comprising an amino acid sequence Gln-Lys-Leu-Val-Phe-Phe; SEQ ID NO:8).

Detailed Description Text (82):
In another specific embodiment, the compound comprises the formula: A-Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 -Xaa.sub.5 -Xaa.sub.6 -Xaa.sub.7 -Xaa.sub.8 -B (e.g., a modified form of A.beta..sub.15-21, comprising an acid sequence Gln-Lys-Leu-Val-Phe-Phe-Ala; SEQ ID NO:7).

Detailed Description Text (83):
In another specific embodiment, the compound comprises the formula: A-Xaa.sub.1 -Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 -Xaa.sub.5 -Xaa.sub.6 -Xaa.sub.7 -B (e.g., a modified form of A.beta..sub.14-20, comprising an amino acid sequence His-Gln-Lys-Leu-Val-Phe-Phe; SEQ ID NO:6).

Detailed Description Text (84):
In another specific embodiment, the compound comprises the formula: A-Xaa.sub.1 -Xaa.sub.2 -Xaa.sub.3 -Xaa.sub.4 -Xaa.sub.5 -Xaa.sub.6 -Xaa.sub.7 -Xaa.sub.8 -B (e.g., a modified form of A.beta..sub.14-21, comprising an amino acid sequence His-Gln-Lys-Leu-Val-Phe-Phe-Ala; SEQ ID NO:5).

Detailed Description Text (86):
In further experiments to delineate subregions of A.beta. upon which an A.beta. aggregation core domain can be modeled (the results of which are described in Example 11), it was demonstrated that a modulator compound having inhibitory activity can comprise as few as three A.beta. amino acids residues (e.g., Val-Phe-Phe, which corresponds to A.beta..sub.8-20 or Phe-Phe-Ala, which corresponds to A.beta..sub.19-21). The results also demonstrated that a modulator compound having a modulating group at its carboxy-terminus is effective at inhibiting A.beta. aggregation. Still further, the results demonstrated that the cholyl group, as a modulating group, can be manipulated while maintaining the inhibitory activity of the compounds and that an iodotyrosyl can be substituted for phenylalanine (e.g., at position 19 or 20 of the A.beta. sequence) while maintaining the ability of the compound to inhibit A.beta. aggregation.

Detailed Description Text (87):
Still further, the results demonstrated that compounds with inhibitory activity can be created using amino acids residues that are derived from the A.beta. sequence in the region of about positions 17-21 but wherein the amino acid sequence is rearranged or has a substitution with a non-A.beta.-derived amino acid. Examples of such compounds include PPI-426, in which the sequence of A.beta..sub.17-21 (LVFFA SEQ ID NO:11) has been rearranged (FFVLA SEQ ID NO:21), PPI-372, in which the sequence of A.beta..sub.16-20 (KLVFF SEQ ID NO:10) has been rearranged (FKFVL SEQ ID NO:29), and PPI-388, -389 and -390, in which the sequence of A.beta..sub.17-21 (LVFFA SEQ ID NO:11) has been substituted at position 17, 18 or 19, respectively, with an alanine residue (AVFFA SEQ ID NO:25 for PPI-388, LAFFA SEQ ID NO:13 for PPI-389 and LVAFA SEQ ID NO:33 for PPI-390). The inhibitory activity of these compounds indicate that the presence in the compound of an amino acid sequence directly corresponding to a portion of A.beta. is not essential for inhibitory activity, but rather suggests that maintenance of the hydrophobic nature of this core region, by inclusion of amino acid residues such as phenylalanine, valine, leucine, regardless of their precise order, can be sufficient for inhibition of A.beta. aggregation. Accordingly, an A.beta. aggregation core domain can be designed based on the direct A.beta. amino acid sequence or can be designed

based on a rearranged A.beta. sequence which maintains the hydrophobicity of the A.beta. subregion, e.g., the region around positions 17-20. This region of A.beta. contains the amino acid residues Leu, Val and Phe. Accordingly, preferred A.beta. aggregation core domains are composed of at least three amino acid structures (as that term is defined hereinbefore, including amino acid derivatives, analogues and mimetics), wherein at least two of the amino acid structures are, independently, either a leucine structure, a valine structure or a phenylalanine structure (as those terms are defined hereinbefore, including derivatives, analogues and mimetics).

Detailed Description Text (88):
Thus, in another embodiment, the invention provides a .beta.-amyloid modulator compound comprising a formula: ##STR9## wherein Xaa.sub.1, Xaa.sub.2 and Xaa.sub.3 are each amino acid structures and at least two of Xaa.sub.1, Xaa.sub.2 and Xaa.sub.3 are, independently, selected from the group consisting of a leucine structure, a phenylalanine structure and a valine structure;

Detailed Description Text (89):
Y, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.a, wherein Xaa is any amino acid structure and a is an integer from 1 to 15;

Detailed Description Text (90):
Z, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.b, wherein Xaa is any amino acid structure and b is an integer from 1 to 15; and

Detailed Description Text (92):
Xaa.sub.1, Xaa.sub.2, Xaa.sub.3, Y, Z, A and n being selected such that the compound modulates the aggregation or inhibits the neurotoxicity of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides.

Detailed Description Text (93):
Preferably, the compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. In preferred embodiments, Xaa.sub.1 and Xaa.sub.2 are each phenylalanine structures or Xaa.sub.2 and Xaa.sub.3 are each phenylalanine structures. "n" can be, for example, an integer between 1 and 5, whereas "a" and "b" can be, for example, integers between 1 and 5. The modifying group "A" preferably comprises a cyclic, heterocyclic or polycyclic group. More preferably, A contains a cis-decalin group, such as cholanoyl structure or a cholyl group. In other embodiments, A can comprise a biotin-containing group, a diethylene-triaminepentaacetyl group, a (-)-menthoxyacetyl group, a fluorescein-containing group or an N-acetylneuraminyl group. In yet other embodiments, the compound may promotes aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides, may be further modified to alter a pharmacokinetic property of the compound or may be further modified to label the compound with a detectable substance.

Detailed Description Text (94):
In another embodiment, the invention provides a .beta.-amyloid modulator compound comprising a formula:

Detailed Description Text (95):
wherein Xaa.sub.1, Xaa.sub.2 and Xaa.sub.3 are each amino acid structures and at least two of Xaa.sub.1, Xaa.sub.2 and Xaa.sub.3 are, independently, selected from the group consisting of a leucine structure, a phenylalanine structure and a valine structure;

Detailed Description Text (96):
Y, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.a, wherein Xaa is any amino acid structure and a is an integer from 1 to 15;

Detailed Description Text (97):
Z, which may or may not be present, is a peptidic structure having the formula (Xaa).sub.b, wherein Xaa is any amino acid structure and b is an integer from 1 to 15; and

Detailed Description Text (99):
Xaa.sub.1, Xaa.sub.2, Xaa.sub.3, Y, Z, A and B being selected such that the compound modulates the aggregation or inhibits the neurotoxicity of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides.

Detailed Description Text (100):
Preferably, the compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. In preferred embodiments, Xaa.sub.1 and Xaa.sub.2 are each phenylalanine structures or Xaa.sub.2 and Xaa.sub.3 are each phenylalanine structures. In one subembodiment, the compound comprises the formula:

Detailed Description Text (102):
"n" can be, for example, an integer between 1 and 5, whereas "a" and "b" can be, for example, integers between 1 and 5. The modifying group "A" preferably comprises a cyclic, heterocyclic or polycyclic group. More preferably, A contains a cis-decalin group, such as cholanoyl structure or a cholyl group In other embodiments, A can comprise a biotin-containing group, a diethylene-triaminepentaacetyl group, a (-)-menthoxyacetyl group, a fluorescein-containing group or an N-acetylneuraminyl group. In yet other embodiments, the compound may promote aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides, may be further modified to alter a pharmacokinetic property of the compound or may be further modified to label the compound with a detectable substance.

Detailed Description Text (103):
In preferred specific embodiments, the invention provides a .beta.-amyloid modulator compound comprising a modifying group attached directly or indirectly to a peptidic structure, wherein the peptidic structure comprises amino acid structures having an amino acid sequence selected from the group consisting of His-Gln-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:5), His-Gln-Lys-Leu-Val-Phe-Phe (SEQ ID NO:6), Gln-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:7), Gln-Lys-Leu-Val-Phe-Phe (SEQ ID NO:8), Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:9), Lys-Leu-Val-Phe-Phe (SEQ ID NO:10), Leu-Val-Phe-Phe-Ala (SEQ ID NO:11), Leu-Val-Phe-Phe (SEQ ID NO:12), Leu-Ala-Phe-Phe-Ala (SEQ ID NO:13), Val-Phe-Phe (SEQ ID NO:19), Phe-Phe-Ala (SEQ ID NO:20), Phe-Phe-Val-Leu-Ala (SEQ ID NO:21), Leu-Val-Phe-Phe-Lys (SEQ ID NO:22), Leu-Val-Iodotyrosine-Phe-Ala (SEQ ID NO:23), Val-Phe-Phe-Ala (SEQ ID NO:24), Ala-Val-Phe-Phe-Ala (SEQ ID NO:25), Leu-Val-Phe-Iodotyrosine-Ala (SEQ ID NO:26), Leu-Val-Phe-Phe-Ala-Glu (SEQ ID NO:27), Phe-Phe-Val-Leu (SEQ ID NO:28), Phe-Lys-Phe-Val-Leu (SEQ ID NO:29), Lys-Leu-Val-Ala-Phe (SEQ ID NO:30), Lys-Leu-Val-Phe-Phe-.beta.Ala (SEQ ID NO:31) and Leu-Val-Phe-Phe-DAla (SEQ ID NO:32).

Detailed Description Text (105):
The modulator compounds of the invention can be incorporated into pharmaceutical compositions (described further in subsection V below) and can be used in detection and treatment methods as described further in subsection VI below.

Detailed Description Text (107):
Within a modulator compound of the invention, a peptidic structure (such as an A.beta. derived peptide, or an A.beta. aggregation core domain, or an amino acid sequence corresponding to a rearranged A.beta. aggregation core domain) is coupled directly or indirectly to at least one modifying group (abbreviated as MG). In one embodiment, a modulator compounds of the invention comprising an aggregation core domain coupled to a modifying group, the compound can be illustrated schematically as MG-ACD. The term "modifying group" is intended to include structures that are directly attached to the peptidic structure (e.g., by covalent coupling), as well as those that are indirectly attached to the peptidic structure (e.g., by a stable non-covalent association or by covalent coupling to additional amino acid residues, or mimetics, analogues or derivatives thereof, which may flank the A.beta.-derived peptidic structure). For example, the modifying group can be coupled to the amino-terminus or carboxy-terminus of an A.beta.-derived peptidic structure, or to a peptidic or peptidomimetic region flanking the core domain. Alternatively, the modifying group can be coupled to a side chain of at least one amino acid residue of an A.beta.-derived peptidic structure, or to a peptidic or peptidomimetic region flanking the core domain (e.g., through the epsilon amino group of a lysyl residue(s), through the carboxyl group of an aspartic acid residue(s) or a glutamic acid residue(s), through a hydroxy group of a tyrosyl residue(s), a serine residue(s) or a threonine residue(s) or other suitable reactive group on an amino acid side chain). Modifying groups covalently coupled to the peptidic structure can be attached by means and using methods well known in the art for linking chemical structures, including, for example, amide, alkylamino, carbamate or urea bonds.

Detailed Description Text (108):
The term "modifying group" is intended to include groups that are not naturally coupled to natural A.beta. peptides in their native form. Accordingly, the term "modifying group" is not intended to include hydrogen. The modifying group(s) is selected such

that the modulator compound alters, and preferably inhibits, aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides or inhibits the neurotoxicity of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. Although not intending to be limited by mechanism, the modifying group(s) of the modulator compounds of the invention is thought to function as a key pharmacophore which is important for conferring on the modulator the ability to disrupt A.beta. polymerization.

Detailed Description Text (112):
A preferred polycyclic group is a group containing a cis-decalin structure. Although not intending to be limited by mechanism, it is thought that the "bent" conformation conferred on a modifying group by the presence of a cis-decalin structure contributes to the efficacy of the modifying group in disrupting A.beta. polymerization. Accordingly, other structures which mimic the "bent" configuration of the cis-decalin structure can also be used as modifying groups. An example of a cis-decalin containing structure that can be used as a modifying group is a cholanoyl structure, such as a cholyl group. For example, a modulator compound can be modified at its amino terminus with a cholyl group by reacting the aggregation core domain with cholic acid, a bile acid, as described in Example 4 (the structure of cholic acid is illustrated in FIG. 2). Moreover, a modulator compound can be modified at its carboxy terminus with a cholyl group according to methods known in the art (see e.g., Wess, G. et al. (1993) Tetrahedron Letters, 34:817-822; Wess, G. et al. (1992) Tetrahedron Letters 33:195-198; and Kramer, W. et al. (1992) J. Biol. Chem. 267:18598-18604). Cholyl derivatives and analogues can also be used as modifying groups. For example, a preferred cholyl derivative is Aic (3-(O-aminoethyl-iso)-cholyl), which has a free amino group that can be used to further modify the modulator compound (e.g., a chelation group for .sup.99m Tc can be introduced through the free amino group of Aic). As used herein, the term "cholanoyl structure" is intended to include the cholyl group and derivatives and analogues thereof, in particular those which retain a four-ring cis-decalin configuration. Examples of cholanoyl structures include groups derived from other bile acids, such as deoxycholic acid, lithocholic acid, ursodeoxycholic acid, chenodeoxycholic acid and hyodeoxycholic acid, as well as other related structures such as cholanic acid, bufalin and resibufogenin (although the latter two compounds are not preferred for use as a modifying group). Another example of a cis-decalin containing compound is 5.beta.-cholestan-3.alpha.-ol (the cis-decalin isomer of (+)-dihydrocholesterol). For further description of bile acid and steroid structure and nomenclature, see Nes, W. R. and McKean, M. L. Biochemistry of Steroids and Other Isopentanoids, University Park Press, Baltimore, Md., Chapter 2.

Detailed Description Text (113):
In addition to cis-decalin containing groups, other polycyclic groups may be used as modifying groups. For example, modifying groups derived from steroids or .beta.-lactams may be suitable modifying groups. Moreover, non-limiting examples of some additional cyclic, heterocyclic or polycyclic compounds which can be used to modify an A.beta.-derived peptidic structure are shown schematically in FIG. 2. In one embodiment, the modifying group is a "biotinyl structure", which includes biotinyl groups and analogues and derivatives thereof (such as a 2-iminobiotinyl group). In another embodiment, the modifying group can comprise a "fluorescein-containing group", such as a group derived from reacting an A.beta.-derived peptidic structure with 5-(and 6-)-carboxyfluorescein, succinimidyl ester or fluorescein isothiocyanate. In various other embodiments, the modifying group(s) can comprise an N-acetylneuraminyl group, a trans-4-cotininecarboxyl group, a 2-imino-1-imidazolidineacetyl group, an (S)-(-)-indoline-2-carboxyl group, a (-)-menthoxyacetyl group, a 2-norbornaneacetyl group, a .gamma.-oxo-5-acenaphthenebutyryl, a (-)-2-oxo-4-thiazolidinecarboxyl group, a tetrahydro-3-furoyl group, a 2-iminobiotinyl group, a diethylenetriaminepentaacetyl group, a 4-morpholinecarbonyl group, a 2-thiopheneacetyl group or a 2-thiophenesulfonyl group.

Detailed Description Text (116):
Yet another type of modifying group is a compound that contains a non-natural amino acid that acts as a beta-turn mimetic, such as a dibenzofuran-based amino acid described in Tsang, K. Y. et al. (1994) J. Am. Chem. Soc. 116:3988-4005; Diaz, H. and Kelly, J. W. (1991) Tetrahedron Letters 41:5725-5728; and Diaz, H. et al. (1992) J. Am. Chem. Soc. 114:8316-8318. An example of such a modifying group is a peptide-aminoethyldibenzofuranyl-proprionic acid (Adp) group (e.g., DDIIL-Adp; SEQ ID NO:34). This type of modifying group further can comprise one or more N-methyl peptide bonds to introduce additional steric hindrance to the aggregation of natural .beta.-AP when compounds of this type interact with natural .beta.-AP.

Detailed Description Text (118):
A .beta.-amyloid modulator compound of the invention can be further modified to alter the specific properties of the compound while retaining the ability of the compound to alter A.beta. aggregation and inhibit A.beta. neurotoxicity. For example, in one embodiment, the compound is further modified to alter a pharmacokinetic property of the compound, such as in vivo stability or half-life. In another embodiment, the compound is further modified to label the compound with a detectable substance. In yet another embodiment, the compound is further modified to couple the compound to an additional therapeutic moiety. Schematically, a modulator of the invention comprising an A.beta. aggregation core domain coupled directly or indirectly to at least one modifying group can be illustrated as MG-ACD, whereas this compound which has been further modified to alter the properties of the modulator can be illustrated as MG-ACD-CM, wherein CM represents an additional chemical modification.

Detailed Description Text (119):
To further chemically modify the compound, such as to alter the pharmacokinetic properties of the compound, reactive groups can be derivatized. For example, when the modifying group is attached to the amino-terminal end of the aggregation core domain, the carboxy-terminal end of the compound can be further modified. Preferred C-terminal modifications include those which reduce the ability of the compound to act as a substrate for carboxypeptidases. Examples of preferred C-terminal modifiers include an amide group, an ethylamide group and various non-natural amino acids, such as D-amino acids and .beta.-alanine. Alternatively, when the modifying group is attached to the carboxy-terminal end of the aggregation core domain, the amino-terminal end of the compound can be further modified, for example, to reduce the ability of the compound to act as a substrate for aminopeptidases.

Detailed Description Text (120):
A modulator compound can be further modified to label the compound by reacting the compound with a detectable substance. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, .beta.-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include .sup.14 C, .sup.123 I, .sup.124 I, .sup.125 I, .sup.131 I, .sup.99m Tc, .sup.35 S or .sup.3 H. In a preferred embodiment, a modulator compound is radioactively labeled with .sup.14 C, either by incorporation of .sup.14 C into the modifying group or one or more amino acid structures in the modulator compound. Labeled modulator compounds can be used to assess the in vivo pharmacokinetics of the compounds, as well as to detect A.beta. aggregation, for example for diagnostic purposes. A.beta. aggregation can be detected using a labeled modulator compound either in vivo or in an in vitro sample derived from a subject.

Detailed Description Text (121):
Preferably, for use as an in vivo diagnostic agent, a modulator compound of the invention is labeled with radioactive technetium or iodine. Accordingly, in one embodiment, the invention provides a modulator compound labeled with technetium, preferably .sup.99m Tc. Methods for labeling peptide compounds with technetium are known in the art (see e.g., U.S. Pat. Nos. 5,443,815, 5,225,180 and 5,405,597, all by Dean et al.; Stepniak-Biniakiewicz, D., et al. (1992) J. Med. Chem. 35:274-279; Fritzberg, A. R., et al. (1988) Proc. Natl. Acad. Sci. USA 85:4025-4029; Baidoo, K. E., et al. (1990) Cancer Res. Suppl. 50:799s-803s; and Regan, L. and Smith, C. K. (1995) Science 270:980-982). A modifying group can be chosen that provides a site at which a chelation group for .sup.99m Tc can be introduced, such as the Aic derivative of cholic acid, which has a free amino group (see Example 11). In another embodiment, the invention provides a modulator compound labeled with radioactive iodine. For example, a phenylalanine residue within the A.beta. sequence (such as Phe.sub.19 or Phe.sub.20) can be substituted with radioactive iodotyrosyl (see Example 11). Any of the various isotopes of radioactive iodine can be incorporated to create a diagnostic agent. Preferably, .sup.123 I (half-life=13.2 hours) is used for whole body scintigraphy, .sup.124 I (half life=4 days) is used for positron emission tomography (PET), .sup.125 I (half life=60 days) is used for metabolic turnover studies and 131I (half life=8 days) is used for whole body counting and delayed low resolution imaging studies.

Detailed Description Text (122):
Furthermore, an additional modification of a modulator compound of the invention can

serve to confer an additional therapeutic property on the compound. That is, the additional chemical modification can comprise an additional functional moiety. For example, a functional moiety which serves to break down or dissolve amyloid plaques can be coupled to the modulator compound. In this form, the MG-ACD portion of the modulator serves to target the compound to A.beta. peptides and disrupt the polymerization of the A.beta. peptides, whereas the additional functional moiety serves to break down or dissolve amyloid plaques after the compound has been targeted to these sites.

Detailed Description Text (123):
In an alternative chemical modification, a .beta.-amyloid compound of the invention is prepared in a "prodrug" form, wherein the compound itself does not modulate A.beta. aggregation, but rather is capable of being transformed, upon metabolism in vivo, into a .beta.-amyloid modulator compound as defined herein. For example, in this type of compound, the modulating group can be present in a prodrug form that is capable of being converted upon metabolism into the form of an active modulating group. Such a prodrug form of a modifying group is referred to herein as a "secondary modifying group." A variety of strategies are known in the art for preparing peptide prodrugs that limit metabolism in order to optimize delivery of the active form of the peptide-based drug (see e.g., Moss, J. (1995) in Peptide-Based Drug Design: Controlling Transport and Metabolism, Taylor, M. D. and Amidon, G. L. (eds), Chapter 18. Additionally strategies have been specifically tailored to achieving CNS delivery based on "sequential metabolism" (see e.g., Bodor, N., et al. (1992) Science 257:1698-1700; Prokai, L., et al. (1994) J. Am. Chem. Soc. 116:2643-2644; Bodor, N. and Prokai, L. (1995) in Peptide-Based Drug Design: Controlling Transport and Metabolism, Taylor, M. D. and Amidon, G. L. (eds), Chapter 14. In one embodiment of a prodrug form of a modulator of the invention, the modifying group comprises an alkyl ester to facilitate blood-brain barrier permeability.

Detailed Description Text (124):
Modulator compounds of the invention can be prepared by standard techniques known in the art. The peptide component of a modulator composed, at least in part, of a peptide, can be synthesized using standard techniques such as those described in Bodansky, M. Principles of Peptide Synthesis, Springer Verlag, Berlin (1993) and Grant, G. A. (ed.). Synthetic Peptides: A User's Guide, W. H. Freeman and Company, New York (1992). Automated peptide synthesizers are commercially available (e.g., Advanced ChemTech Model 396; Milligen/Biosearch 9600). Additionally, one or more modulating groups can be attached to the A.beta.-derived peptidic component (e.g., an A.beta. aggregation core domain) by standard methods, for example using methods for reaction through an amino group (e.g., the alpha-amino group at the amino-terminus of a peptide), a carboxyl group (e.g., at the carboxy terminus of a peptide), a hydroxyl group (e.g., on a tyrosine, serine or threonine residue) or other suitable reactive group on an amino acid side chain (see e.g., Greene, T. W. and Wuts, P. G. M. Protective Groups in Organic Synthesis, John Wiley and Sons, Inc., New York (1991)). Exemplary syntheses of preferred .beta. amyloid modulators is described further in Examples 1, 4 and 11.

Detailed Description Text (126):
Another aspect of the invention pertains to a method for selecting a modulator of .beta.-amyloid aggregation. In the method, a test compound is contacted with natural .beta. amyloid peptides, the aggregation of the natural .beta.-AP is measured and a modulator is selected based on the ability of the test compound to alter the aggregation of the natural .beta.-AP (e.g., inhibit or promote aggregation). In a preferred embodiment, the test compound is contacted with a molar excess amount of the natural .beta.-AP. The amount and/or rate of natural .beta.-AP aggregation in the presence of the test compound can be determined by a suitable assay indicative of .beta.-AP aggregation, as described herein (see e.g., Examples 2, 5 and 6).

Detailed Description Text (127):
In a preferred assay, the natural .beta.-AP is dissolved in solution in the presence of the test compound and aggregation of the natural .beta.-AP is assessed in a nucleation assay (see Example 6) by assessing the turbidity of the solution over time, as measured by the apparent absorbance of the solution at 405 nm (described further in Example 6; see also Jarrett et al. (1993) Biochemistry 32:4693-4697). In the absence of a .beta.-amyloid modulator, the A.sub.405 nm of the solution typically stays relatively constant during a lag time in which the .beta.-AP remains in solution, but then the A.sub.405 nm of the solution rapidly increases as the .beta.-AP aggregates and comes out of solution, ultimately reaching a plateau level (i.e., the A.sub.405 nm of the solution exhibits sigmoidal kinetics over time). In contrast, in the presence of a test compound that inhibits .beta.-AP aggregation, the A.sub.405 nm, of the solution is reduced compared to when the modulator is absent. Thus, in the presence of the

inhibitory modulator, the solution may exhibit an increased lag time, a decreased slope of aggregation and/or a lower plateau level compared to when the modulator is absent. This method for selecting a modulator of .beta.-amyloid polymerization can similarly be used to select modulators that promote .beta.-AP aggregation. Thus, in the presence of a modulator that promotes .beta.-AP aggregation, the A.sub.405 nm of the solution is increased compared to when the modulator is absent (e.g., the solution may exhibit an decreased lag time, increase slope of aggregation and/or a higher plateau level compared to when the modulator is absent).

Detailed Description Text (128):
Another assay suitable for use in the screening method of the invention, a seeded extension assay, is also described further in Example 6. In this assay, .beta.-AP monomer and an aggregated .beta.-AP "seed" are combined, in the presence and absence of a test compound, and the amount of .beta.-fibril formation is assayed based on enhanced emission of the dye Thioflavine T when contacted with .beta.-AP fibrils. Moreover, .beta.-AP aggregation can be assessed by electron microscopy (EM) of the .beta.-AP preparation in the presence or absence of the modulator. For example, .beta. amyloid fibril formation, which is detectable by EM, is reduced in the presence of a modulator that inhibits .beta.-AP aggregation (i.e., there is a reduced amount or number of .beta.-fibrils in the presence of the modulator), whereas .beta. fibril formation is increased in the presence of a modulator that promotes .beta.-AP aggregation (i.e., there is an increased amount or number of .beta.-fibrils in the presence of the modulator).

Detailed Description Text (131):
Another aspect of the invention pertains to pharmaceutical compositions of the .beta.-amyloid modulator compounds of the invention. In one embodiment, the composition includes a .beta. amyloid modulator compound in a therapeutically or prophylactically effective amount sufficient to alter, and preferably inhibit, aggregation of natural .beta.-amyloid peptides, and a pharmaceutically acceptable carrier. In another embodiment, the composition includes a .beta. amyloid modulator compound in a therapeutically or prophylactically effective amount sufficient to inhibit the neurotoxicity of natural .beta.-amyloid peptides, and a pharmaceutically acceptable carrier. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as reduction or reversal or .beta.-amyloid deposition and/or reduction or reversal of A.beta. neurotoxicity. A therapeutically effective amount of modulator may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the modulator to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental effects of the modulator are outweighed by the therapeutically beneficial effects. The potential neurotoxicity of the modulators of the invention can be assayed using the cell-based assay described in Examples 3 and 10 and a therapeutically effective modulator can be selected which does not exhibit significant neurotoxicity. In a preferred embodiment, a therapeutically effective amount of a modulator is sufficient to alter, and preferably inhibit, aggregation of a molar excess amount of natural .beta.-amyloid peptides. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, such as preventing or inhibiting the rate of .beta.-amyloid deposition and/or A.beta. neurotoxicity in a subject predisposed to .beta.-amyloid deposition. A prophylactically effective amount can be determined as described above for the therapeutically effective amount. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Detailed Description Text (132):
One factor that may be considered when determining a therapeutically or prophylactically effective amount of a .beta. amyloid modulator is the concentration of natural .beta.-AP in a biological compartment of a subject, such as in the cerebrospinal fluid (CSF) of the subject. The concentration of natural .beta.-AP in the CSF has been estimated at 3 nM (Schwartzman, (1994) Proc. Natl. Acad. Sci. USA 91:8368-8372). A non-limiting range for a therapeutically or prophylactically effective amounts of a .beta. amyloid modulator is 0.01 nM-10 .mu.M. It is to be noted that dosage values may vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit

the scope or practice of the claimed composition.

Detailed Description Text (133):
The amount of active compound in the composition may vary according to factors such as the disease state, age, sex, and weight of the individual, each of which may affect the amount of natural .beta.-AP in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

Detailed Description Text (135):
Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and gelatin. Moreover, the modulators can be administered in a time release formulation, for example in a composition which includes a slow release polymer. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). Many methods for the preparation of such formulations are patented or generally known to those skilled in the art.

Detailed Description Text (136):
Sterile injectable solutions can be prepared by incorporating the active compound (e.g., .beta.-amyloid modulator) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Detailed Description Text (137):
A modulator compound of the invention can be formulated with one or more additional compounds that enhance the solubility of the modulator compound. Preferred compounds to be added to formulations to enhance the solubility of the modulators are cyclodextrin derivatives, preferably hydroxypropyl-.gamma.-cyclodextrin. Drug delivery vehicles containing a cyclodextrin derivative for delivery of peptides to the central nervous system are described in Bodor, N., et al. (1992) Science 257:1698-1700. For the .beta.-amyloid modulators described herein, inclusion in the formulation of hydroxypropyl-.gamma.-cyclodextrin at a concentration 50-200 mM increases the aqueous solubility of the compounds. In addition to increased solubility, inclusion of a cyclodextrin derivative in the formulation may have other beneficial effects, since .beta.-cyclodextrin itself has been reported to interact with the A.beta. peptide and inhibit fibril formation in vitro (Camilleri, P., et al. (1994) FEBS Letters 341:256-258. Accordingly, use of a modulator compound of the invention in combination with a cyclodextrin derivative may result in greater inhibition of A.beta. aggregation

than use of the modulator alone. Chemical modifications of cyclodextrins are known in the art (Hanessian, S., et al. (1995) J. Org. Chem. 60:4786-4797). In addition to use as an additive in a pharmaceutical composition containing a modulator of the invention, cyclodextrin derivatives may also be useful as modifying groups and, accordingly, may also be covalently coupled to an A.beta. peptide compound to form a modulator compound of the invention.

Detailed Description Text (138):
In another embodiment, a pharmaceutical composition comprising a modulator of the invention is formulated such that the modulator is transported across the blood-brain barrier (BBB). Various strategies known in the art for increasing transport across the BBB can be adapted to the modulators of the invention to thereby enhance transport of the modulators across the BBB (for reviews of such strategies, see e.g., Pardridge, W. M. (1994) Trends in Biotechnol. 12:239-245; Van Bree, J. B. et al. (1993) Pharm. World Sci. 15:2-9; and Pardridge, W. M. et al. (1992) Pharmacol. Toxicol. 71:3-10). In one approach, the modulator is chemically modified to form a prodrug with enhanced transmembrane transport. Suitable chemical modifications include covalent linking of a fatty acid to the modulator through an amide or ester linkage (see e.g., U.S. Pat. No. 4,933,324 and PCT Publication WO 89/07938, both by Shashoua; U.S. Pat. No. 5,284,876 by Hesse et al.; Toth, I. et al. (1 994) J. Drug Target. 2:217-239; and Shashoua, V. E. et al. (1984) J. Med. Chem. 27:659-664) and glycating the modulator (see e.g., U.S. Pat. No. 5,260,308 by Poduslo et al.). Also, N-acylamino acid derivatives may be used in a modulator to form a "lipidic" prodrug (see e.g., U.S. Pat. No. 5,112,863 by Hashimoto et al.).

Detailed Description Text (139):
In another approach for enhancing transport across the BBB, a peptidic or peptidomimetic modulator is conjugated to a second peptide or protein, thereby forming a chimeric protein, wherein the second peptide or protein undergoes absorptive-mediated or receptor-mediated transcytosis through the BBB. Accordingly, by coupling the modulator to this second peptide or protein, the chimeric protein is transported across the BBB. The second peptide or protein can be a ligand for a brain capillary endothelial cell receptor ligand. For example, a preferred ligand is a monoclonal antibody that specifically binds to the transferrin receptor on brain capillary endothelial cells (see e.g., U.S. Pat. Nos. 5,182,107 and 5,154,924 and PCT Publications WO 93/10819 and WO 95/02421, all by Friden et al.). Other suitable peptides or proteins that can mediate transport across the BBB include histones (see e.g., U.S. Pat. No. 4,902,505 by Pardridge and Schimmel) and ligands such as biotin, folate, niacin, pantothenic acid, riboflavin, thiamin, pryridoxal and ascorbic acid (see e.g., U.S. Pat. Nos. 5,416,016 and 5,108,921, both by Heinstein). Additionally, the glucose transporter GLUT-1 has been reported to transport glycopeptides (L-serinyl-.beta.-D-glucoside analogues of [Met5]enkephalin) across the BBB (Polt, R. et al. (1994) Proc. Natl. Acad. Sci. USA 91:7114-1778). Accordingly, a modulator compound can be coupled to such a glycopeptide to target the modulator to the GLUT-1 glucose transporter. For example, a modulator compound which is modified at its amino terminus with the modifying group Aic (3-(O-aminoethyl-iso)-cholyl, a derivative of cholic acid having a free amino group) can be coupled to a glycopeptide through the amino group of Aic by standard methods. Chimeric proteins can be formed by recombinant DNA methods (e.g., by formation of a chimeric gene encoding a fusion protein) or by chemical crosslinking of the modulator to the second peptide or protein to form a chimeric protein. Numerous chemical crosslinking agents are known in the (e.g., commercially available from Pierce, Rockford Ill.). A crosslinking agent can be chosen which allows for high yield coupling of the modulator to the second peptide or protein and for subsequent cleavage of the linker to release bioactive modulator. For example, a biotin-avidin-based linker system may be used.

Detailed Description Text (143):
In another embodiment, a pharmaceutical composition of the invention is provided as a packaged formulation. The packaged formulation may include a pharmaceutical composition of the invention in a container and printed instructions for administration of the composition for treating a subject having a disorder associated with .beta.-amyloidosis, e.g. Alzheimer's disease.

Detailed Description Text (145):
Another aspect of the invention pertains to methods for altering the aggregation or inhibiting the neurotoxicity of natural .beta.-amyloid peptides. In the methods of the invention, natural .beta. amyloid peptides are contacted with a .beta. amyloid modulator such that the aggregation of the natural .beta. amyloid peptides is altered or the neurotoxicity of the natural .beta. amyloid peptides is inhibited. In a

preferred embodiment, the modulator inhibits aggregation of the natural .beta. amyloid peptides. In another embodiment, the modulator promotes aggregation of the natural .beta. amyloid peptides. Preferably, aggregation of a molar excess amount of .beta.-AP, relative to the amount of modulator, is altered upon contact with the modulator.

Detailed Description Text (146):
In the method of the invention, natural .beta. amyloid peptides can be contacted with a modulator either in vitro or in vivo. Thus, the term "contacted with" is intended to encompass both incubation of a modulator with a natural .beta.-AP preparation in vitro and delivery of the modulator to a site in vivo where natural .beta.-AP is present. Since the modulator compound interacts with natural .beta.-AP, the modulator compounds can be used to detect natural .beta.-AP, either in vitro or in vivo. Accordingly, one use of the modulator compounds of the invention is as diagnostic agents to detect the presence of natural .beta.-AP, either in a biological sample or in vivo in a subject. Furthermore, detection of natural .beta.-AP utilizing a modulator compound of the invention further can be used to diagnose amyloidosis in a subject. Additionally, since the modulator compounds of the invention disrupt .beta.-AP aggregation and inhibit .beta.-AP neurotoxicity, the modulator compounds also are useful in the treatment of disorders associated with .beta.-amyloidosis, either prophylactically or therapeutically. Accordingly, another use of the modulator compounds of the invention is as therapeutic agents to alter aggregation and/or neurotoxicity of natural .beta.-AP.

Detailed Description Text (147):
In one embodiment, a modulator compound of the invention is used in vitro, for example to detect and quantitate natural .beta.-AP in sample (e.g., a sample of biological fluid). To aid in detection, the modulator compound can be modified with a detectable substance. The source of natural .beta.-AP used in the method can be, for example, a sample of cerebrospinal fluid (e.g., from an AD patient, an adult susceptible to AD due to family history, or a normal adult). The natural .beta.-AP sample is contacted with a modulator of the invention and aggregation of the .beta.-AP is measured, such as by as assay described in Examples 2, 5 and 6. Preferably, the nucleation assay and/or seeded extension assay described in Example 6 is used. The degree of aggregation of the .beta.-AP sample can then be compared to that of a control sample(s) of a known concentration of .beta.-AP, similarly contacted with the modulator and the results can be used as an indication of whether a subject is susceptible to or has a disorder associated with .beta.-amyloidosis. Moreover, .beta.-AP can be detected by detecting a modulating group incorporated into the modulator. For example, modulators incorporating a biotin compound as described herein (e.g., an amino-terminally biotinylated .beta.-AP peptide) can be detected using a streptavidin or avidin probe which is labeled with a detectable substance (e.g., an enzyme, such as peroxidase). Detection of natural .beta.-AP aggregates mixed with a modulator of the invention using a probe that binds to the modulating group (e.g., biotin/streptavidin) is described further in Example 2.

Detailed Description Text (148):
In another embodiment, a modulator compound of the invention is used in vivo to detect, and, if desired, quantitate, natural .beta.-AP deposition in a subject, for example to aid in the diagnosis of .beta. amyloidosis in the subject. To aid in detection, the modulator compound can be modified with a detectable substance, preferably .sup.99m Tc or radioactive iodine (described further above), which can be detected in vivo in a subject. The labeled .beta.-amyloid modulator compound is administered to the subject and, after sufficient time to allow accumulation of the modulator at sites of amyloid deposition, the labeled modulator compound is detected by standard imaging techniques. The radioactive signal generated by the labeled compound can be directly detected (e.g., whole body counting), or alternatively, the radioactive signal can be converted into an image on an autoradiograph or on a computer screen to allow for imaging of amyloid deposits in the subject. Methods for imaging amyloidosis using radiolabeled proteins are known in the art. For example, serum amyloid .beta. component (SAP), radiolabeled with either .sup.123 I or .sup.99m Tc, has been used to image systemic amyloidosis (see e.g., Hawkins, P. N. and Pepys, M. B. (1995) Eur. J Nucl. Med. 22:595-599). Of the various isotypes of radioactive iodine, preferably .sup.123 I (half-life=13.2 hours) is used for whole body scintigraphy, .sup.124 I (half life=4 days) is used for positron emission tomography (PET), .sup.125 I (half life=60 days) is used for metabolic turnover studies and .sup.131 I (half life=8 days) is used for whole body counting and delayed low resolution imaging studies. Analogous to studies using radiolabeled SAP, a labeled modulator compound of the invention can be delivered to a subject by an appropriate route (e.g., intravenously, intraspinally, intracerebrally) in a single bolus, for example containing 100 .mu.g of labeled compound carrying approximately 180 MBq of radioactivity.

Detailed Description Text (149):
The invention provides a method for detecting the presence or absence of natural .beta.-amyloid peptides in a biological sample, comprising contacting a biological sample with a compound of the invention and detecting the compound bound to natural .beta.-amyloid peptides to thereby detect the presence or absence of natural .beta.-amyloid peptides in the biological sample. In one embodiment, the .beta.-amyloid modulator compound and the biological sample are contacted in vitro. In another embodiment, the .beta.-amyloid modulator compound is contacted with the biological sample by administering the .beta.-amyloid modulator compound to a subject. For in vivo administration, preferably the compound is labeled with radioactive technetium or radioactive iodine.

Detailed Description Text (150):
The invention also provides a method for detecting natural .beta.-amyloid peptides to facilitate diagnosis of a .beta.-amyloidogenic disease, comprising contacting a biological sample with the compound of the invention and detecting the compound bound to natural .beta.-amyloid peptides to facilitate diagnosis of a .beta.-amyloidogenic disease. In one embodiment, the .beta.-amyloid modulator compound and the biological sample are contacted in vitro. In another embodiment, the .beta.-amyloid modulator compound is contacted with the biological sample by administering the .beta.-amyloid modulator compound to a subject. For in vivo administration, preferably the compound is labeled with radioactive technetium or radioactive iodine. Preferably, use of the method facilitates diagnosis of Alzheimer's disease.

Detailed Description Text (151):
In another embodiment, the invention provides a method for altering natural .beta.-AP aggregation or inhibiting .beta.-AP neurotoxicity, which can be used prophylactically or therapeutically in the treatment or prevention of disorders associated with .beta. amyloidosis, e.g., Alzheimer's Disease. As demonstrated in Example 10, modulator compounds of the invention reduce the toxicity of natural .beta.-AP aggregates to cultured neuronal cells. Moreover, the modulators not only reduce the formation of neurotoxic aggregates but also have the ability to reduce the neurotoxicity of preformed A.beta. fibrils. Accordingly, the modulator compounds of the invention can be used to inhibit or prevent the formation of neurotoxic A.beta. fibrils in subjects (e.g., prophylactically in a subject predisposed to .beta.-amyloid deposition) and can be used to reverse .beta.-amyloidosis therapeutically in subjects already exhibiting .beta.-amyloid deposition.

Detailed Description Text (152):
A modulator of the invention is contacted with natural .beta. amyloid peptides present in a subject (e.g., in the cerebrospinal fluid or cerebrum of the subject) to thereby alter the aggregation of the natural .beta.-AP and/or inhibit the neurotoxicity of the natural .beta.-APs. A modulator compound alone can be administered to the subject, or alternatively, the modulator compound can be administered in combination with other therapeutically active agents (e.g., as discussed above in subsection IV). When combination therapy is employed, the therapeutic agents can be coadministered in a single pharmaceutical composition, coadministered in separate pharmaceutical compositions or administered sequentially.

Detailed Description Text (153):
The modulator may be administered to a subject by any suitable route effective for inhibiting natural .beta.-AP aggregation in the subject, although in a particularly preferred embodiment, the modulator is administered parenterally, most preferably to the central nervous system of the subject. Possible routes of CNS administration include intraspinal administration and intracerebral administration (e.g., intracerebrovascular administration). Alternatively, the compound can be administered, for example, orally, intraperitoneally, intravenously or intramuscularly. For non-CNS administration routes, the compound can be administered in a formulation which allows for transport across the BBB. Certain modulators may be transported across the BBB without any additional further modification whereas others may need further modification as described above in subsection IV.

Detailed Description Text (155):
The method of the invention for altering .beta.-AP aggregation in vivo, and in particular for inhibiting .beta.-AP aggregation, can be used therapeutically in diseases associated with abnormal .beta. amyloid aggregation and deposition to thereby slow the rate of .beta. amyloid deposition and/or lessen the degree of .beta. amyloid deposition, thereby ameliorating the course of the disease. In a preferred embodiment,

the method is used to treat Alzheimer's disease (e.g, sporadic or familial AD, including both individuals exhibiting symptoms of AD and individuals susceptible to familial AD). The method can also be used prophylactically or therapeutically to treat other clinical occurrences of, amyloid deposition, such as in Down's syndrome individuals and in patients with hereditary cerebral hemorrhage with amyloidosis-Dutch-type (HCHWA-D). While inhibition of .beta.-AP aggregation is a preferred therapeutic method, modulators that promote .beta.-AP aggregation may also be useful therapeutically by allowing for the sequestration of .beta.-AP at sites that do not lead to neurological impairment.

Detailed Description Text (156):
Additionally, abnormal accumulation of .beta.-amyloid precursor protein in muscle fibers has been implicated in the pathology of sporadic inclusion body myositis (IBM) (Askanas, V. et al. (1996) Proc. Natl. Acad Sci. USA 93:1314-1319; Askanas, V. et al. (1995) Current Opinion in Rheumatology 7:486-496). Accordingly, the modulators of the invention can be used prophylactically or therapeutically in the treatment of disorders in which .beta.-AP, or APP, is abnormally deposited at non-neurological locations, such as treatment of IBM by delivery of the modulators to muscle fibers.

Detailed Description Text (157):
VII. Unmodified A.beta. Peptides that Inhibit Aggregation of Natural .beta.-AP

Detailed Description Text (158):
In addition to the .beta.-amyloid modulators described hereinbefore in which an A.beta. peptide is coupled to a modifying group, the invention also provides .beta.-amyloid modulators comprised of an unmodified A.beta. peptide. It has now been discovered that certain portions of natural .beta.-AP can alter aggregation of natural .beta.-APs when contacted with the natural .beta.-APs (see Example 12). Accordingly, these unmodified A.beta. peptides comprise a portion of the natural .beta.-AP sequence (i.e., a portion of .beta.AP.sub.1-39, .beta.AP.sub.1-40, .beta.AP.sub.1-42 and .beta.AP.sub.1-43). In particular these unmodified A.beta. peptides have at least one amino acid deletion compared to .beta.AP.sub.1-39, the shortest natural .beta.-AP, such that the compound alters aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. In various embodiments, these unmodified peptide compounds can promote aggregation of natural .beta.-amyloid peptides, or, more preferably, can inhibit aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. Even more preferably, the unmodified peptide compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with a molar excess amount of natural .beta.-amyloid peptides (e.g., a 10-fold, 33-fold or 100-fold molar excess amount of natural .beta.-AP).

Detailed Description Text (159):
As discussed above, the unmodified peptide compounds of the invention comprise an amino acid sequence having at least one amino acid deletion compared to the amino acid sequence of .beta.AP.sub.1-39. Alternatively, the unmodified peptide compound can have at least five, ten, fifteen, twenty, twenty-five, thirty or thirty-five amino acids deleted compared to .beta.AP.sub.1-39. Still further the unmodified peptide compound can have 1-5, 1-10, 1-15, 1-20, 1-25, 1-30 or 1-35 amino acids deleted compared to .beta.AP.sub.1-39. The amino acid deletion(s) may occur at the amino-terminus, the carboxy-terminus, an internal site, or a combination thereof, of the .beta.-AP sequence. Accordingly, in one embodiment, an unmodified peptide compound of the invention comprises an amino acid sequence which has at least one internal amino acid deleted compared to .beta.AP.sub.1-39. Alternatively, the unmodified peptide compound can have at least five, ten, fifteen, twenty, twenty-five, thirty or thirty-five internal amino acids deleted compared to .beta.AP.sub.1-39. Still further the unmodified peptide compound can have 1-5, 1-10, 1-15, 1-20, 1-25, 1-30 or 1-35 internal amino acids deleted compared to .beta.AP.sub.1-39. For peptides with internal deletions, preferably the peptide has an amino terminus corresponding to amino acid residue 1 of natural .beta.AP and a carboxy terminus corresponding to residue 40 of natural .beta.AP and has one or more internal .beta.-AP amino acid residues deleted (i.e., a non-contiguous A.beta. peptide).

Detailed Description Text (160):
In another embodiment, the unmodified peptide compound comprises an amino acid sequence which has at least one N-terminal amino acid deleted compared to .beta.AP.sub.1-39. Alternatively, the unmodified peptide compound can have at least five, ten, fifteen, twenty, twenty-five, thirty or thirty-five N-terminal amino acids deleted compared to .beta.AP.sub.1-39. Still further the unmodified peptide compound can have 1-5, 1-10, 1-15, 1-20, 1-25, 1-30 or 1-35 N-terminal amino acids deleted compared to

.beta.AP.sub.1-39.

Detailed Description Text (161):
In yet another embodiment, the unmodified peptide compound comprises an amino acid sequence which has at least one C-terminal amino acid deleted compared to .beta.AP.sub.1-39 Alternatively, the unmodified peptide compound can have at least five, ten, fifteen, twenty, twenty-five, thirty or thirty-five C-terminal amino acids deleted compared to .beta.AP.sub.1-39. Still further the unmodified peptide compound can have 1-5, 1-10, 1-15, 1-20, 1-25, 1-30 or 1-35 C-terminal amino acids deleted compared to .beta.AP.sub.1-39.

Detailed Description Text (162):
In addition to deletion of amino acids as compared to .beta.AP.sub.1-39, the peptide compound can have additional non-.beta.-AP amino acid residues added to it, for example, at the amino terminus, the carboxy-terminus or at an internal site. In one embodiment, the peptide compound has at least one non-.beta.-amyloid peptide-derived amino acid at its N-terminus. Alternatively, the compound can have, for example, 1-3, 1-5, 1-7, 1-10, 1-15 or 1-20 non-.beta.-amyloid peptide-derived amino acid at its N-terminus. In another embodiment, the peptide compound has at least one non-.beta.-amyloid peptide-derived amino acid at its C-terminus. Alternatively, the compound can have, for example, 1-3, 1-5, 1-7, 1-10, 1-15 or 1-20 non-.beta.-amyloid peptide-derived amino acid at its C-terminus.

Detailed Description Text (163):
In specific preferred embodiments, an unmodified peptide compound of the invention comprises A.beta..sub.6-20 (the amino acid sequence of which is shown in SEQ ID NO:4), A.beta..sub.16-30 (the amino acid sequence of which is shown in SEQ ID NO:14), A.beta..sub.1-20, 26-40 (the amino acid sequence of which is shown in SEQ ID NO:15) or EEVVHHHHQQ-.beta.AP.sub.16-40 (the amino acid sequence of which is shown in SEQ ID NO:16). In the nomenclature used herein, .beta.AP.sub.1-20, 26-40 represents .beta.AP.sub.1-40 in which the internal amino acid residues 21-25 have been deleted.

Detailed Description Text (164):
An unmodified peptide compound of the invention can be chemically synthesized using standard techniques such as those described in Bodansky, M. Principles of Peptide Synthesis, Springer Verlag, Berlin (1993) and Grant, G. A. (ed.). Synthetic Peptides: A User's Guide, W. H. Freeman and Company, New York (1992). Automated peptide synthesizers are commercially available (e.g., Advanced ChemTech Model 396; Milligen/Biosearch 9600). Alternatively, unmodified peptide compounds can be prepared according to standard recombinant DNA techniques using a nucleic acid molecule encoding the peptide. A nucleotide sequence encoding the peptide can be determined using the genetic code and an oligonucleotide molecule having this nucleotide sequence can be synthesized by standard DNA synthesis methods (e.g., using an automated DNA synthesizer). Alternatively, a DNA molecule encoding an unmodified peptide compound can be derived from the natural .beta.-amyloid precursor protein gene or cDNA (e.g., using the polymerase chain reaction and/or restriction enzyme digestion) according to standard molecular biology techniques.

Detailed Description Text (165):
Accordingly, the invention further provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a .beta.-amyloid peptide compound, the .beta.-amyloid peptide compound comprising an amino acid sequence having at least one amino acid deletion compared to .beta.AP.sub.1-39 such that the .beta.-amyloid peptide compound alters aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules and RNA molecules and may be single-stranded or double-stranded, but preferably is double-stranded DNA. The isolated nucleic acid encodes a peptide wherein one or more amino acids are deleted from the N-terminus, C-terminus and/or an internal site of .beta.AP.sub.1-39, as discussed above. In yet other embodiments, the isolated nucleic acid encodes a peptide compound having one or more amino acids deleted compared to .beta.AP.sub.1-39 and further having at least one non-.beta.-AP derived amino acid residue added to it, for example, at the amino terminus, the carboxy-terminus or at an internal site. In specific preferred embodiments, an isolated nucleic acid molecule of the invention encodes .beta.AP.sub.6-20, .beta.AP.sub.16-30, .beta.AP.sub.1-20, 26-40 or EEVVHHHHQQ-.beta.AP.sub.16-40 (SEQ ID NO:16).

Detailed Description Text (166):
To facilitate expression of a peptide compound in a host cell by standard recombinant

DNA techniques, the isolated nucleic acid encoding the peptide is incorporated into a recombinant expression vector. Accordingly, the invention also provides recombinant expression vectors comprising the nucleic acid molecules of the invention. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" or simply "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors, which serve equivalent functions.

Detailed Description Text (167):
In the recombinant expression vectors of the invention, the nucleotide sequence encoding the peptide compound are operatively linked to one or more regulatory sequences, selected on the basis of the host cells to be used for expression. The term "operably linked" is intended to mean that the sequences encoding the peptide compound are linked to the regulatory sequence(s) in a manner that allows for expression of the peptide compound. The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell, those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences) and those that direct expression in a regulatable manner (e.g., only in the presence of an inducing agent). It will be appreciated by those skilled in the art that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed, the level of expression of peptide compound desired, etc. The expression vectors of the invention can be introduced into host cells thereby to produce peptide compounds encoded by nucleic acids as described herein.

Detailed Description Text (170):
A recombinant expression vector comprising a nucleic acid encoding a peptide compound that alters aggregation of natural .beta.-AP can be introduced into a host cell to thereby produce the peptide compound in the host cell. Accordingly, the invention also provides host cells containing the recombinant expression vectors of the invention. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. A host cell may be any prokaryotic or eukaryotic cell. For example, a peptide compound may be expressed in bacterial cells such as E. coli, insect cells, yeast or mammalian cells. Preferably, the peptide compound is expressed in mammalian cells. In a preferred embodiment, the peptide compound is expressed in mammalian cells in vivo in a mammalian subject to treat amyloidosis in the subject through gene therapy (discussed further below). Preferably, the .beta.-amyloid peptide compound encoded by the recombinant expression vector is secreted from the host cell upon being expressed in the host cell.

Detailed Description Text (171):
Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, electroporation, microinjection and viral-mediated transfection. Suitable methods for transforming or transfecting host cells can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory

press (1989)), and other laboratory manuals. Methods for introducing DNA into mammalian cells in vivo are also known in the art and can be used to deliver the vector DNA to a subject for gene therapy purposes (discussed further below).

Detailed Description Text (172):
For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker may be introduced into a host cell on the same vector as that encoding the peptide compound or may be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

Detailed Description Text (173):
A nucleic acid of the invention can be delivered to cells in vivo using methods known in the art, such as direct injection of DNA, receptor-mediated DNA uptake or viral-mediated transfection. Direct injection has been used to introduce naked DNA into cells in vivo (see e.g., Acsadi et al. (1991) Nature 332:815-818; Wolff et al. (1990) Science 247:1465-1468). A delivery apparatus (e.g., a "gene gun") for injecting DNA into cells in vivo can be used. Such an apparatus is commercially available (e.g., from BioRad). Naked DNA can also be introduced into cells by complexing the DNA to a cation, such as polylysine, which is coupled to a ligand for a cell-surface receptor (see for example Wu, G. and Wu, C. H. (1988) J. Biol. Chem. 263:14621; Wilson el al. (1992) J. Biol. Chem. 267:963-967; and U.S. Pat. No. 5,166,320). Binding of the DNA-ligand complex to the receptor facilitates uptake of the DNA by receptor-mediated endocytosis. Additionally, a DNA-ligand complex linked to adenovirus capsids which naturally disrupt endosomes, thereby releasing material into the cytoplasm can be used to avoid degradation of the complex by intracellular lysosomes (see for example Curiel el al. (1991) Proc. Natl. Acad. Sci. USA 88:8850; Cristiano et al. (1993) Proc. Natl. Acad. Sci. USA 90:2122-2126).

Detailed Description Text (174):
Defective retroviruses are well characterized for use in gene transfer for gene therapy purposes (for a review see Miller, A. D. (1990) Blood 76:271). Protocols for producing recombinant retroviruses and for infecting cells in vitro or in vivo with such viruses can be found in Current Protocols in Molecular Biology, Ausubel, F. M. et al. (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM which are well known to those skilled in the art. Examples of suitable packaging virus lines include .psi.Crip, .psi.Cre, .psi.2 and .psi.Am. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, endothelial cells, lymphocytes, myoblasts, hepatocytes, bone marrow cells, in vitro and/or in vivo (see for example Eglitis, et al. (1985) Science 230:1395-1398; Danos and Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:6460-6464; Wilson et al. (1988) Proc. Natl. Acad. Sci. USA 85:3014-3018; Armentano et al. (1990) Proc. Natl. Acad. Sci. USA 87:6141-6145; Huber et al. (1991) Proc. Natl. Acad. Sci. USA 88:8039-8043; Ferry et al. (1991) Proc. Natl. Acad. Sci. USA 88:8377-8381; Chowdhury et al. (1991) Science 254:1802-1805; van Beusechem et al. (1992) Proc. Natl. Acad. Sci. USA 89:7640-7644; Kay et al. (1992) Human Gene Therapy 3:641-647; Dai et al. (1992) Proc. Natl. Acad. Sci. USA 89:10892-10895; Hwu et al. (1993) J. Immunol. 150:4104-4115; U.S. Pat. No. 4,868,116; U.S. Pat. No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573).

Detailed Description Text (176):
Adeno-associated virus (AAV) can also be used for delivery of DNA for gene therapy purposes. AAV is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle. (For a review see Muzyczka et al. Curr. Topics in Micro. and Immunol. (1992) 158:97-129). It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (see for example Flotte et al. (1992) Am. J. Respir. Cell. Mol. Biol. 7:349-356; Samulski et al. (1989) J. Virol. 63:3822-3828; and McLaughlin et al. (1989) J. Virol. 62:1963-1973). Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate. An AAV vector such as that described in Tratschin et al. (1985) Mol. Cell. Biol. 5:3251-3260 can be used to introduce DNA into cells. A variety of nucleic

acids have been introduced into different cell types using AAV vectors (see for example Hermonat et al. (1984) Proc. Natl. Acad. Sci. USA 81:6466-6470; Tratschin et al. (1985) Mol. Cell. Biol. 4:2072-2081; Wondisford et al. (1988) Mol. Endocrinol. 2:32-39; Tratschin et al. (1984) J. Virol. 51:611-619; and Flotte et al. (1993) J. Biol. Chem. 268:3781-3790).

Detailed Description Text (177):
The invention provides a method for treating a subject for a disorder associated with .beta.-amyloidosis, comprising administering to the subject a recombinant expression vector encoding a .beta.-amyloid peptide compound, the compound comprising an amino acid sequence having at least one amino acid deletion compared to .beta.AP.sub.1-39, such that the .beta.-amyloid peptide compound is synthesized in the subject and the subject is treated for a disorder associated with .beta.-amyloidosis. Preferably, the disorder is Alzheimer's disease. In one embodiment the recombinant expression vector directs expression of the peptide compound in neuronal cells. In another embodiment, the recombinant expression vector directs expression of the peptide compound in glial cells. In yet another embodiment, the recombinant expression vector directs expression of the peptide compound in fibroblast cells.

Detailed Description Text (178):
General methods for gene therapy are known in the art. See for example, U.S. Pat. No. 5,399,346 by Anderson et al. A biocompatible capsule for delivering genetic material is described in PCT Publication WO 95/05452 by Baetge et al. Methods for grafting genetically modified cells to treat central nervous system disorders are described in U.S. Pat. No. 5,082,670 and in PCT Publications WO 90/06757 and WO 93/10234, all by Gage et al. Isolation and/or genetic modification of multipotent neural stem cells or neuro-derived fetal cells are described in PCT Publications WO 94/02593 by Anderson et al., WO 94/16718 by Weiss et al., and WO 94/23754 by Major et al. Fibroblasts transduced with genetic material are described in PCT Publication WO 89/02468 by Mulligan et al. Adenovirus vectors for transfering genetic material into cells of the central nervous system are described in PCT Publication WO 94/08026 by Kahn et al. Herpes simplex virus vectors suitable for treating neural disorders are described in PCT Publications WO 94/04695 by Kaplitt and WO 90/09441 by Geller et al. Promoter elements of the glial fibrillary acidic protein that can confer astrocyte specific expression on a linked gene or gene fragment, and which thus can be used for expression of A.beta. peptides specifically in astrocytes, is described in PCT Publication WO 93/07280 by Brenner et al. Furthermore, alternative to expression of an A.beta. peptide to modulate amyloidosis, an antisense oligonucleotide that is complementary to a region of the .beta.-amyloid precursor protein mRNA corresponding to the peptides described herein can be expressed in a subject to modulate amyloidosis. General methods for expressing antisense oligonucleotides to modulate nervous system disorders are described in PCT Publication WO 95/09236.

Detailed Description Text (179):
Alternative to delivery by gene therapy, a peptide compound of the invention comprising an amino acid sequence having at least one amino acid deletion compared to .beta.AP.sub.1-39 can be delivered to a subject by directly administering the peptide compound to the subject as described further herein for the modified peptide compounds of the invention. The peptide compound can be formulated into a pharmaceutical composition comprising a therapeutically effective amount of the .beta.-amyloid peptide compound and a pharmaceutically acceptable carrier. The peptide compound can be contacted with natural .beta.-amyloid peptides with a .beta.-amyloid peptide compound such that aggregation of the natural .beta.-amyloid peptides is inhibited. Moreover, the peptide compound can be administered to the subject in a therapeutically effective amount such that the subject is treated for a disorder associated with .beta.-amyloidosis, such as Alzheimer's disease.

Detailed Description Text (181):
Although the invention has been illustrated hereinbefore with regard to A.beta. peptide compounds, the principles described, involving attachment of a modifying group(s) to a peptide compound, are applicable to any amyloidogenic protein or peptide as a means to create a modulator compound that modulates, and preferably inhibits, amyloid aggregation. Accordingly, the invention provides modulator compounds that can be used to treat amyloidosis in a variety of forms and clinical settings.

Detailed Description Text (182):
Amyloidosis is a general term used to describe pathological conditions characterized by the presence of amyloid. Amyloid is a general term referring to a group of diverse but specific extracellular protein deposits which are seen in a number of different

diseases. Though diverse in their occurrence, all amyloid deposits have common morphologic properties, stain with specific dyes (e.g., Congo red), and have a characteristic red-green birefringent appearance in polarized light after staining. They also share common ultrastructural features and common x-ray diffraction and infrared spectra. Amyloidosis can be classified clinically as primary, secondary, familial and/or isolated. Primary amyloid appears de novo without any preceding disorder. Secondary amyloid is that form which appears as a complication of a previously existing disorder. Familial amyloid is a genetically inherited form found in particular geographic populations. Isolated forms of amyloid are those that tend to involve a single organ system.

Detailed Description Text (183):
Different amyloids are characterized by the type of protein(s) or peptide(s) present in the deposit. For example, as described hereinbefore, amyloid deposits associated with Alzheimer's disease comprise the .beta.-amyloid peptide and thus a modulator compound of the invention for detecting and/or treating Alzheimer's disease is designed based on modification of the .beta.-amyloid peptide. The identities of the protein(s) or peptide(s) present in amyloid deposits associated with a number of other amyloidogenic diseases have been elucidated. Accordingly, modulator compounds for use in the detection and/or treatment of these other amyloidogenic diseases can be prepared in a similar fashion to that described herein for .beta.-AP-derived modulators. In vitro assay systems can be established using an amyloidogenic protein or peptide which forms fibrils in vitro, analogous to the A.beta. assays described herein. Modulators can be identified using such assay systems, based on the ability of the modulator to disrupt the .beta.-sheet structure of the fibrils. Initially, an entire amyloidogenic protein can be modified or, more preferably, a peptide fragment thereof that is known to form fibrils in vitro can be modified (e.g., analogous to A.beta.1-40 described herein). Amino acid deletion and substitution analyses can then be performed on the modified protein or peptide (analogous to the studies described in the Examples) to delineate an aggregation core domain that is sufficient, when modified, to disrupt fibril formation.

Detailed Description Text (185):
Transthyretin (TTR)--Amyloids containing transthyretin occur in familial amyloid polyneuropathy (Portuguese, Japanese and Swedish types), familial amyloid cardiomyopathy (Danish type), isolated cardiac amyloid and systemic senile amyloidosis. Peptide fragments of transthyretin have been shown to form amyloid fibrils in vitro. For example, TTR 10-20 and TTR 105-115 form amyloid-like fibrils in 20-30% acetonitrile/water at room temperature (Jarvis, J. A., et al. (1994) Int. J. Pept. Protein Res. 44:388-398). Moreover, familial cardiomyopathy (Danish type) is associated with mutation of Leu at position 111 to Met, and an analogue of TTR 105-115 in which the wildtype Leu at position 111 has been substituted with Met (TTR 105-115Met111) also forms amyloid-like fibrils in vitro (see e.g., Hermansen, L. F., et al. (1995) Eur. J. Biochem. 227:772-779; Jarvis et al. supra). Peptide fragments of TTR that form amyloid fibrils in vitro are also described in Jarvis, J. A., et al. (1993) Biochem. Biophys. Res. Commun. 192:991-998 and Gustavsson, A., et al. (1991) Biochem. Biophys. Res. Commun. 175:1159-1164. A peptide fragment of wildtype or mutated transthyretin that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of familial amyloid polyneuropathy (Portuguese, Japanese and Swedish types), familial amyloid cardiomyopathy (Danish type), isolated cardiac amyloid or systemic senile amyloidosis.

Detailed Description Text (186):
Prion Protein (PrP)--Amyloids in a number of spongiform encephalopathies, including scrapie in sheep, bovine spongiform encephalopathy in cows and Creutzfeldt-Jakob disease (CJ) and Gerstmann-Straussler-Scheinker syndrome (GSS) in humans, contain PrP. Limited proteolysis of PrPSc (the prion protein associated with scrapie) leads to a 27-30 kDa fragment (PrP27-30) that polymerizes into rod-shaped amyloids (see e.g., Pan, K. M., et al. (1993) Proc. Natl. Acad. Sci. USA 90:10962-10966; Gasset, M., et al. (1993) Proc. Natl. Acad. Sci. USA 90:1-5). Peptide fragments of PrP from humans and other mammals have been shown to form amyloid fibrils in vitro. For example, polypeptides corresponding to sequences encoded by normal and mutant alleles of the PRNP gene (encoding the precursor of the prion protein involved in CJ), in the regions of codon 178 and codon 200, spontaneously form amyloid fibrils in vitro (see e.g., Goldfarb, L. G., et al. (1993) Proc. Natl. Acad. Sci. USA 90:4451-4454). A peptide encompassing residues 106-126 of human PrP has been reported to form straight fibrils similar to those extracted from GSS brains, whereas a peptide encompassing residues 127-147 of human PrP has been reported to form twisted fibrils resembling scrapie-associated fibrils (Tagliavini, F., et al. (1993) Proc. Natl. Acad. Sci. USA

90:9678-9682). Peptides of Syrian hamster PrP encompassing residues 109-122, 113-127, 113-120, 178-191 or 202-218 have been reported to form amyloid fibrils, with the most amyloidogenic peptide being Ala-Gly-Ala-Ala-Ala-Ala-Gly-Ala (SEQ ID NO:17), which corresponds to residues 113-120 of Syrian hamster PrP but which is also conserved in PrP from other species (Gasset, M., et al. (1992) Proc. Natl. Acad. Sci. USA 89:10940-10944). A peptide fragment of PrP that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of scrapie, bovine spongiform encephalopathy, Creutzfeldt-Jakob disease or Gerstmann-Straussler-Scheinker syndrome.

Detailed Description Text (187):
Islet Amyloid Polypeptide (IAPP, also known as amylin)--Amyloids containing IAPP occur in adult onset diabetes and insulinoma. IAPP is a 37 amino acid polypeptide formed from an 89 amino acid precursor protein (see e.g., Betsholtz, C., et al. (1989) Exp. Cell. Res. 183:484-493; Westermark, P., et al. (1987) Proc. Natl. Acad. Sci. USA 84:3881-3885). A peptide corresponding to IAPP residues 20-29 has been reported to form amyloid-like fibrils in vitro, with residues 25-29, having the sequence Ala-Ile-Leu-Ser-Ser (SEQ ID NO:18), being strongly amyloidogenic (Westermark, P., et al. (1990) Proc. Natl. Acad. Sci. USA 87:5036-5040; Glenner, G. G., et al. (1988) Biochem. Biophys. Res. Commun. 155:608-614). A peptide fragment of IAPP that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of adult onset diabetes or insulinoma.

Detailed Description Text (188):
Atrial Natriuretic Factor (ANF)--Amyloids containing ANF are associated with isolated atrial amyloid (see e.g., Johansson, B., et al. (1987) Biochem. Biophys. Res. Commun. 148:1087-1092). ANF corresponds to amino acid residues 99-126 (proANF99-126) of the ANF prohormone (proANP1-126) (Pucci, A., et al. (1991) J. Pathol. 165:235-241). ANF, or a fragment thereof, that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of isolated atrial amyloid.

Detailed Description Text (189):
Kappa or Lambda Light Chain--Amyloids containing kappa or lambda light chains are associated idiopathic (primary) amyloidosis, myeloma or macroglobulinemia-associated amyloidosis, and primary localized cutaneous nodular amyloidosis associated with Sjogren's syndrome. The structure of amyloidogenic kappa and lambda light chains, including amino acid sequence analysis, has been characterized (see e.g., Buxbaum, J. N., et al. (1990) Ann. Intern. Med. 112:455-464; Schormann, N., et al. (1995) Proc. Natl. Acad. Sci. USA 92:9490-9494; Hurle, M. R., et al. (1994) Proc. Natl. Acad. Sci. USA 91:5446-5450; Liepnieks, J. J., et al. (1990) Mol. Immunol. 27:481-485; Gertz, M. A., et al. (1985) Scand. J. Immunol. 22:245-250; Inazumi, T., et al. (1994) Dermatology 189:125-128). Kappa or lambda light chains, or a peptide fragment thereof that forms amyloid fibrils, can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of idiopathic (primary) amyloidosis, myeloma or macroglobulinemia-associated amyloidosis or primary localized cutaneous nodular amyloidosis associated with Sjogren's syndrome.

Detailed Description Text (190):
Amyloid A--Amyloids containing the amyloid A protein (AA protein), derived from serum amyloid A, are associated with reactive (secondary) amyloidosis (see e.g., Liepnieks, J. J., et al. (1995) Biochim. Biophys. Acta 1270:81-86), familial Mediterranean Fever and familial amyloid nephropathy with urticaria and deafness (Muckle-Wells syndrome) (see e.g., Linke, R. P., et al. (1983) Lab. Invest. 48:698-704). Recombinant human serum amyloid A forms amyloid-like fibrils in vitro (Yamada, T., et al. (1994) Biochim. Biophys. Acta 1226:323-329) and circular dichroism studies revealed a predominant .beta. sheet/turn structure (McCubbin, W. D., et al. (1988) Biochem J. 256:775-783). Serum amyloid A, amyloid A protein or a fragment thereof that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of reactive (secondary) amyloidosis, familial Mediterranean Fever and familial amyloid nephropathy with urticaria and deafness (Muckle-Wells syndrome).

Detailed Description Text (191):
Cystatin C--Amyloids containing a variant of cystatin C are associated with hereditary cerebral hemorrhage with amyloidosis of Icelandic type. The disease is associated with a leucine to glycine mutation at position 68 and cystatin C containing this mutation aggregates in vitro (Abrahamson, M. and Grubb, A. (1994) Proc. Natl. Acad. Sci. USA

91:1416-1420). Cystatin C or a peptide fragment thereof that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of hereditary cerebral hemorrhage with amyloidosis of Icelandic type.

Detailed Description Text (192):
.beta.2 microglobulin--Amyloids containing .beta.2 microglobulin (.beta.2M) are a major complication of long term hemodialysis (see e.g., Stein, G., et al. (1994) Nephrol. Dial. Transplant. 9:48-50; Floege, J., et al. (1992) Kidney Int. Suppl. 38:S78-S85; Maury, C. P. (1990) Rheumatol. Int. 10:1-8). The native .beta.2M protein has been shown to form amyloid fibrils in vitro (Connors, L. H., et al. (1985) Biochem. Biophys. Res. Commun. 131:1063-1068; Ono, K., et al. (1994) Nephron 66:404-407). .beta.2M, or a peptide fragment thereof that forms amyloid fibrils, can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of amyloidosis associated with long term hemodialysis.

Detailed Description Text (193):
Apolipoprotein A-I (ApoA-I)--Amyloids containing variant forms of ApoA-I have been found in hereditary non-neuropathic systemic amyloidosis (familial amyloid polyneuropathy III). For example, N-terminal fragments (residues 1-86, 1-92 and 1-93) of an ApoA-I variant having a Trp to Arg mutation at position 50 have been detected in amyloids (Booth, D. R., et al. (1995) QJM 88:695-702). In another family, a leucine to arginine mutation at position 60 was found (Soutar, A. K., et al. (1992) Proc. Natl. Acad. Sci. USA 89:7389-7393). ApoA-I or a peptide fragment thereof that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of hereditary non-neuropathic systemic amyloidosis.

Detailed Description Text (194):
Gelsolin--Amyloids containing variants of gelsolin are associated with familial amyloidosis of Finnish type. Synthetic gelsolin peptides that have sequence homology to wildtype or mutant gelsolins and that form amyloid fibrils in vitro are reported in Maury, C. P. et al. (1994) Lab. Invest. 70:558-564. A nine residue segment surrounding residue 187 (which is mutated in familial gelsolin amyloidosis) was defined as an amyloidogenic region (Maury, et al., supra; see also Maury, C. P., et al. (1992) Biochem. Biophys. Res. Commun. 183:227-231; Maury, C. P. (1991) J. Clin. Invest. 87:1195-1199). Gelsolin or a peptide fragment thereof that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of familial amyloidosis of Finnish type.

Detailed Description Text (195):
Procalcitonin or calcitonin--Amyloids containing procalcitonin, calcitonin or calcitonin-like immunoreactivity have been detected in amyloid fibrils associated with medullary carcinoma of the thyroid (see e.g., Butler, M. and Khan, S. (1986) Arch. Pathol. Lab. Med. 110:647-649; Sletten, K., et al. (1976) J. Exp. Med. 143:993-998). Calcitonin has been shown to form a nonbranching fibrillar structure in vitro (Kedar, I., et al. (1976) Isr. J. Med. Sci. 12:1137-1140). Procalcitonin, calcitonin or a fragment thereof that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of amyloidosis associated with medullary carcinoma of the thyroid.

Detailed Description Text (196):
Fibrinogen--Amyloids containing a variant form of fibrinogen alpha-chain have been found in hereditary renal amyloidosis. An arginine to leucine mutation at position 554 has been reported in amyloid fibril protein isolated from postmortem kidney of an affected individual (Benson, M. D., et al. (1993) Nature Genetics 3:252-255). Fibrinogen alpha-chain or a peptide fragment thereof that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of fibrinogen-associated hereditary renal amyloidosis.

Detailed Description Text (197):
Lysozyme--Amyloids containing a variant form of lysozyme have been found in hereditary systemic amyloidosis. In one family the disease was associated with a threonine to isoleucine mutation at position 56, whereas in another family the disease was associated with a histidine to aspartic acid mutation at position 67 (Pepys, M. B., et al. (1993) Nature 362:553-557). Lysozyme or a peptide fragment thereof that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of lysozyme-associated hereditary systemic amyloidosis.

Detailed Description Text (198):
This invention is further illustrated by the following examples which should not be construed as limiting. A modulator's ability to alter the aggregation of .beta.-amyloid peptide in the assays described below are predictive of the modulator's ability to perform the same function in vivo. The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated by reference.

Detailed Description Text (200):
Construction of .beta.-Amyloid Modulators

Detailed Description Text (201):
A .beta.-amyloid modulator composed of an amino-terminally biotinylated .beta.-amyloid peptide of the amino acid sequence:

Detailed Description Text (202):
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV (positions 1 to 40 of SEQ ID NO:1) was prepared by solid-phase peptide synthesis using an N.sup..alpha.
-9-fluorenylmethyloxycarbonyl (FMOC)-based protection strategy as follows. Starting with 2.5 mmoles of FMOC-Val-Wang resin, sequential additions of each amino acid were performed using a four-fold excess of protected amino acids, 1-hydroxybenzotriazole (HOBt) and diisopropyl carbodiimide (DIC). Recouplings were performed when necessary as determined by ninhydrin testing of the resin after coupling. Each synthesis cycle was minimally described by a three minute deprotection (25% piperidine/N-methyl-pyrrolidone (NMP)), a 15 minute deprotection, five one minute NMP washes, a 60 minute coupling cycle, five NMP washes and a ninhydrin test. To a 700 mg portion of the fully assembled peptide-resin, biotin (obtained commercially from Molecular Probes, Inc.) was substituted for an FMOC-amino acid was coupled by the above protocol. The peptide was removed from the resin by treatment with trifluoroacetic acid (TFA) (82.5%), water (5%), thioanisole (5%), phenol (5%), ethanedithiol (2.5%) for two hours followed by precipitation of the peptide in cold ether. The solid was pelleted by centrifugation (2400 rpm.times.10 min.), and the ether decanted. It was resuspended in ether, pelleted and decanted a second time. The solid was dissolved in 10% acetic acid and lyophilized to dryness to yield 230 mg of crude biotinylated peptide. 60 mg of the solid was dissolved in 25% acetonitrile (ACN)/0.1% TFA and applied to a C18 reversed phase high performance liquid chromatography (HPLC) column. Biotinyl .beta.AP.sub.1-40 was eluted using a linear gradient of 30-45% acetonitrile/0.1% TFA over 40 minutes. One primary fraction (4 mg) and several side fractions were isolated. The main fraction yielded a mass spectrum of 4556 (matrix-assisted laser desorption ionization-time of flight) which matches the theoretical (4555) for this peptide.

Detailed Description Text (203):
A .beta.-amyloid modulator composed of an amino-terminally biotinylated .beta.-amyloid peptide of the amino acid sequence:

Detailed Description Text (204):
DAEFRHDSGYEVHHQ (positions 1 to 15 of SEQ ID NO:1) was prepared on an Advanced ChemTech Model 396 multiple peptide synthesizer using an automated protocol established by the manufacturer for 0.025 mmole scale synthesis. Double couplings were performed on all cycles using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)/N,N-diisopropylethylamine (DIEA)/HOBt/FMOC-AA in four-fold excess for 30 minutes followed by DIC/HOBt/FMOC-AA in four-fold excess for 45 minutes. The peptide was deprotected and removed from the resin by treatment with TFA/water (95%/5%) for three hours and precipitated with ether as described above. The pellet was resuspended in 10% acetic acid and lyophilized. The material was purified by a preparative HPLC using 15%-40% acetonitrile over 80 minutes on a Vydac C18 column (21.times.250 mm)'. The main isolate eluted as a single symmetrical peak when analyzed by analytical HPLC and yielded the expected molecular weight when analyzed by electrospray mass spectrometry. Result=2052.6 (2052 theoretical).

Detailed Description Text (205):
.beta.-amyloid modulator compounds comprising other regions of the .beta.-AP amino acid sequence (e.g., an A.beta. aggregation core domain) were similarly prepared using the synthesis methods described above. Moreover, modulators comprising other amyloidogenic peptides can be similarly prepared.

Detailed Description Text (207):
Inhibition of .beta.-Amyloid Aggregation by Modulators

Detailed Description Text (208):
The ability of .beta.-amyloid modulators to inhibit the aggregation of natural .beta.-AP when combined with the natural .beta.-AP was examined in a series of aggregation assays. Natural .beta.-AP (.beta.-AP.sub.1-40) was obtained commercially from Bachem (Torrance, Calif.). Amino-terminally biotinylated .beta.-AP modulators were prepared as described in Example 1.

Detailed Description Text (210):
In one assay, .beta.-AP aggregation was measured by determining the increase in turbidity of a solution of natural .beta.-AP over time in the absence or presence of various concentrations of the modulator. Turbidity of the solution was quantitated by determining the optical density at 400 nm (A.sub.400 nm) of the solution over time.

Detailed Description Text (211):
The aggregation of natural .beta.-AP in the absence of modulator was determined as follows. .beta.-AP.sub.1-40 was dissolved in hexafluoro isopropanol (HFIP; Aldrich Chemical Co., Inc.) at 2 mg/ml. Aliquots of the HFIP solution (87 .mu.l) were transferred to individual 10 mm.times.75 mm test tubes. A stream of argon gas was passed through each tube to evaporate the HFIP. To the resulting thin film of peptide, dimethylsulfoxide (DMSO; Aldrich Chemical Co., Inc.) (25 .mu.l) was added to dissolve the peptide. A 2 mm.times.7 mm TEFLON.TM.-coated magnetic stir bar was added to each tube. Buffer (475 .mu.L of 100 mM NaCl, 10 mM sodium phosphate, pH 7.4) was added to the DMSO solution with stirring. The resulting mixture was stirred continuously and the optical density was monitored at 400 nm to observe the formation of insoluble peptide aggregates.

Detailed Description Text (212):
Alternatively, .beta.-AP.sub.1-40 was dissolved in DMSO as described above at 1.6 mM (6.9 mg/ml) and aliquots (25 .mu.l) were added to stirred buffer (475 .mu.l), followed by monitoring of absorbance at 400 nm.

Detailed Description Text (213):
For inhibition studies in which a .beta.-amyloid modulator was dissolved in solution together with the natural .beta.-AP, the modulators were dissolved in DMSO either with or without prior dissolution in HFIP. These compounds were then added to buffer with stirring, followed by addition of .beta.AP.sub.1-40 in DMSO. Alternatively, HFIP solutions of modulators were combined with .beta.-AP.sub.1-40 in HFIP followed by evaporation and redissolution of the mixture in DMSO. Buffer was then added to the DMSO solution to initiate the assay. The amino-terminally biotinylated .beta.-amyloid peptide modulators N-biotinyl-.beta.AP.sub.1-40, and N-biotinyl-.beta.AP.sub.1-15 were tested at concentrations of 1% and 5% in the natural .beta.-AP.sub.1-40 solution.

Detailed Description Text (214):
A representative example of the results is shown graphically in FIG. 1, which depicts the inhibition of aggregation of natural .beta.-AP .sub.1-40 by N-biotinyl-.beta.AP.sub.1-40. In the absence of the modulator, the optical density of the natural .beta.-AP solution showed a characteristic sigmoidal curve, with a lag time prior to aggregation (approximately 3 hours in FIG. 1) in which the A.sub.400 nm was low, followed by rapid increase in the A.sub.400 nm, which quickly reached a plateau level, representing aggregation of the natural .beta. amyloid peptides. In contrast, in the presence of as little as 1% of the N-biotinyl-.beta.AP.sub.1-40 modulator, aggregation of the natural .beta. amyloid peptides was markedly inhibited, indicated by an increase in the lag time, a decrease in the slope of aggregation and a decrease in the plateau level reached for the turbidity of the solution (see FIG. 1). N-biotinyl-.beta.AP.sub.1-40 at a concentration of 5% similarly inhibited aggregation of the natural .beta. amyloid peptide. Furthermore, similar results were observed when N-biotinyl-.beta.AP.sub.1-15 was used as the modulator. These results demonstrate that an N-terminally biotinylated .beta.-AP modulator can effectively inhibit the aggregation of natural .beta. amyloid peptides, even when the natural .beta. amyloid peptides are present at as much as a 100-fold molar excess concentration.

Detailed Description Text (216):
In a second assay, .beta.-AP aggregation was measured using a fluorometric assay essentially as described in Levine, H. (1993) Protein Science 2:404-410. In this assay, the dye thioflavine T (ThT) is contacted with the .beta.-AP solution. Association of ThT with aggregated .beta.-AP, but not monomeric or loosely associated .beta.-AP, gives rise to a new excitation (ex) maximum at 450 nm and an enhanced emission (em) at 482 nm, compared to the 385 nm (ex) and 445 nm (em) for the free dye. .beta.-AP aggregation

was assayed by this method as follows. Aliquots (2.9 .mu.l) of the solutions used in the aggregation assays as described above in section A were removed from the samples and diluted in 200 .mu.l of potassium phosphate buffer (50 mM, pH 7.0) containing thioflavin T (10 .mu.M; obtained commercially from Aldrich Chemical Co., Inc.). Excitation was set at 450 nm and emission was measured at 482 nm. Similar to the results observed with the optical density assay described above in section A, as little as 1% of the N-biotinylated .beta.-AP modulators was effective at inhibiting the aggregation of natural .beta. amyloid peptides using this fluorometric assay.

Detailed Description Text (218):
In a third assay, .beta.-AP aggregation was measured by visualization of the peptide aggregates using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). In this assay, .beta.-AP solutions were allowed to aggregate over a period of time and then aliquots of the reaction were run on a standard SDS-PAGE gel. Typical solution conditions were 200 .mu.M of .beta.-AP.sub.1-40 in PBS at 37.degree. C. for 8 days or 200 .mu.M .beta.-AP.sub.1-40 in 0.1M sodium acetate at 37.degree. C. for 3 days. The peptide aggregates were visualized by Coomassie blue staining of the gel or, for .beta.-AP solutions that included a biotinylated .beta.-AP modulator, by western blotting of a filter prepared from the gel with a streptavidin-peroxidase probe, followed by a standard peroxidase assay. The .beta.-AP aggregates are identifiable as high molecular weight, low mobility bands on the gel, which are readily distinguishable from the low molecular weight, high mobility .beta.-AP monomer or dimer bands.

Detailed Description Text (219):
When natural .beta.-AP.sub.1-40 aggregation was assayed by this method in the absence of any .beta. amyloid modulators, high molecular weight aggregates were readily detectable on the gel. In contrast, when N-biotinyl-.beta.-AP.sub.1-40 modulator self-aggregation was assayed (i.e., aggregation of the N-biotinyl peptide alone, in the absence of any natural .beta.-AP), few if any high molecular weight aggregates were observed, indicating that the ability of the modulator to self-aggregate is significantly reduced compared to natural .beta.-AP. Finally, when aggregation of a mixture of natural .beta.-AP.sub.1-40 and N-biotinylated .beta.-AP.sub.1-40 was assayed by this method, reduced amounts of the peptide mixture associated into high molecular weight aggregates, thus demonstrating that the .beta. amyloid modulator is effective at inhibiting the aggregation of the natural D amyloid peptides.

Detailed Description Text (221):
Neurotoxicity Analysis of .beta.-Amyloid Modulators

Detailed Description Text (222):
The neurotoxicity of the .beta.-amyloid modulators is tested in a cell-based assay using the neuronal precursor cell line PC-12, or primary neuronal cells, and the viability indicator 3,(4,4-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT). (See Shearman, M. S. et al. (1994) Proc. Natl. Acad. Sci. USA 91:1470-1474; Hansen, M. B. et al. (1989) J. Immun. Methods 119:203-210). PC-12 is a rat adrenal pheochromocytoma cell line and is available from the American Type Culture Collection, Rockville, Md. (ATCC CRL 1721). MTT (commercially available from Sigma Chemical Co.) is a chromogenic substrate that is converted from yellow to blue in viable cells, which can be detected spectrophotometrically.

Detailed Description Text (223):
To test the neurotoxicity of a .beta.-amyloid modulator (either alone or combined with natural .beta.-AP), cells first are plated in 96-well plates at 7,000-10,000 cells/well and allowed to adhere by overnight culture at 37.degree. C. Serial dilutions of freshly dissolved or "aged" modulators (either alone or combined with natural .beta.-AP) in phosphate buffered saline (PBS) are added to the wells in triplicate and incubation is continued for two or more days. Aged modulators are prepared by incubating an aqueous solution of the modulator at 37.degree. C. undisturbed for a prolonged period (e.g., five days or more). For the final two hours of exposure of the cells to the modulator preparation, MTT is added to the media to a final concentration of 1 mg/ml and incubation is continued at 37.degree. C. Following the two hour incubation with MTT, the media is removed and the cells are lysed in isopropanol/0:4N HCl with agitation. An equal volume of PBS is added to each well and the absorbance of each well at 570 nm is measured to quantitate viable cells. Alternatively, MTT is solubilized by addition of 50% N,N-dimethyl formamide/20% sodium dodecyl sulfate added directly to the media in the wells and viable cells are likewise quantitated by measuring absorbance at 570 nm. The relative neurotoxicity of a .beta.-amyloid modulator (either alone or in combination with natural .beta.-AP) is determined by comparison to natural .beta.-AP alone (e.g., .beta.1-40, .beta.1-42), which exhibits neurotoxicity in this assay and

thus can serve as a positive control.

Detailed Description Text (225):
Synthesis of Additional Modified .beta.-Amyloid Peptide Compounds

Detailed Description Text (226):
In this example, a series of modified .beta.-APs, having a variety of N-terminal or random side chain modifications were synthesized.

Detailed Description Text (227):
A series of N-terminally modified .beta.-amyloid peptides was synthesized using standard methods. Fully-protected resin-bound peptides corresponding to A.beta.(1-15) and A.beta.(1-40) were prepared as described in Example 1 on Wang resin to eventually afford carboxyl terminal peptide acids. Small portions of each peptide resin (13 and 20 .mu.moles, respectively) were aliquoted into the wells of the reaction block of an Advanced ChemTech Model 396 Multiple Peptide Synthesizer. The N-terminal FMOC protecting group of each sample was removed in the standard manner with 25% piperidine in NMP followed by extensive washing with NMP. The unprotected N-terminal .alpha.-amino group of each peptide-resin sample was modified using one of the following methods:

Detailed Description Text (228):
Method A, coupling of modifying reagents containing free carboxylic acid groups: The modifying reagent (five equivalents) was predissolved in NMP, DMSO or a mixture of these two solvents. HOBT and DIC (five equivalents of each reagent) were added to the dissolved modifier and the resulting solution was added to one equivalent of free-amino peptide-resin. Coupling was allowed to proceed overnight, followed by washing. If a ninhydrin test on a small sample of peptide-resin showed that coupling was not complete, the coupling was repeated using 1-hydroxy-7-azabenzotriazole (HOAt) in place of HOBt.

Detailed Description Text (231):
Method A was used to couple N-acetylneuraminic acid, cholic acid, trans-4-cotininecarboxylic acid, 2-imino-1-imidazolidineacetic acid, (S)-(-)-indoline-2-carboxylic acid, (-)-menthoxyacetic acid, 2-norbornaneacetic acid, .gamma.-oxo-5-acenaphthenebutyric acid, (-)-2-oxo-4-thiazolidinecarboxylic acid, and tetrahydro-3-furoic acid. Method B was used to couple 2-iminobiotin-N-hydroxysuccinimide ester, diethylenetriaminepentaacetic dianhydride, 4-morpholinecarbonyl chloride, 2-thiopheneacetyl chloride, and 2-thiophenesulfonyl chloride.

Detailed Description Text (233):
In addition to the N-fluoresceinyl A.beta. peptides described above, a .beta.-amyloid modulator comprised of random modification of A.beta.(1-40) with fluorescein was prepared. A.beta.(1-40) purchased from Bachem was dissolved in DMSO at approximately 2 mg/mL. 5-(and-6)-Carboxyfluorescein purchased from Molecular Probes was added in a 1.5 molar excess and DIEA was added to make the solution basic to wet pH paper. The reaction was allowed to proceed for 1 hour at room temperature and was then quenched with triethanolamine. The product was added to assays as this crude mixture.

Detailed Description Text (234):
.beta.-amyloid modulator compounds comprising other regions of the .beta.-AP amino acid sequence (e.g., an A.beta. aggregation core domain) were similarly prepared using the synthesis methods described above. Moreover, modulators comprising other amyloidogenic peptides can be similarly prepared.

Detailed Description Text (236):
Identification of Additional .beta.-Amyloid Modulators

Detailed Description Text (237):
In this Example, two assays of A.beta. aggregation were used to identify .beta.-amyloid modulators which can inhibit this process.

Detailed Description Text (242):
For the aggregation assay, each sample was set up in triplicate [50 .mu.l of 200 .mu.M monomer, 125 FU sheared seed (variable quantity dependent on the batch of seed, routinely 3-6 .mu.l), 10 .mu.l of 10.times. inhibitor solution, final volume made up to 100 .mu.l with 1.times. PBS]. Two concentrations of each inhibitor were tested 100 .mu.M and 10 .mu.M, equivalent to a 1:1 and a 1:10 molar ratio of monomer to inhibitor. The controls included an unseeded reaction to confirm that the fresh monomer contained

no seed, and a seeded reaction in the absence of inhibitor, as a reference to compare against putative inhibitors. The assay was incubated at 37.degree. C. for 6 h, taking 2 .mu.l samples hourly for fluorescence measurements. To measure fluorescence, a 2 .mu.l sample of A.beta. was added to 400 .mu.l of Thioflavin-T solution (50 mM Potassium Phosphate 10 mM Thioflavin-T pH 7.5). The samples were vortexed and the fluorescence was read in a 0.5 ml micro quartz cuvette at EX 450 nm and EM 482 nm (Hitachi 4500 Fluorimeter). .beta.-aggregation results in enhanced emission of Thioflavin-T. Accordingly, samples including an effective inhibitor compound exhibit reduced emission as compared to control samples without the inhibitor compound.

Detailed Description Text (246):
Representative results of the static seeded assay and shaken plate assay with preferred .beta.-amyloid modulators are shown below in Table I.

Detailed Description Text (247):
These results indicate that .beta.-APs modified by a wide variety of N-terminal modifying groups are effective at modulating .beta.-amyloid aggregation.

Detailed Description Text (249):
Additional .beta.-Amyloid Aggregation Assays

Detailed Description Text (250):
Most preferably, the ability of .beta.-amyloid modulator compounds to modulate (e.g., inhibit or promote) the aggregation of natural .beta.-AP when combined with the natural .beta.-AP is examined in one or both of the aggregation assays described below. Natural .beta.-AP (.beta.-AP.sub.1-40) for use in the aggregation assays is commercially available from Bachem (Torrance, Calif.).

Detailed Description Text (252):
The nucleation assay is employed to determine the ability of test compounds to alter (e.g. inhibit) the early events in formation of .beta.-AP fibers from monomeric .beta.-AP. Characteristic of a nucleated polymerization mechanism, a lag time is observed prior to nucleation, after which the peptide rapidly forms fibers as reflected in a linear rise in turbidity. The time delay before polymerization of .beta.-AP monomer can be quantified as well as the extent of formation of insoluble fiber by light scattering (turbidity). Polymerization reaches equilibrium when the maximum turbidity reaches a plateau. The turbidity of a solution of natural .beta.-AP in the absence or presence of various concentrations of a .beta.-amyloid modulator compound is determined by measuring the apparent absorbance of the solution at 405 nm (A.sub.405 nm) over time. The threshold of sensitivity for the measurement of turbidity is in the range of 15-20 .mu.M .beta.-AP. A decrease in turbidity over time in the presence of the modulator, as compared to the turbidity in the absence of the modulator, indicates that the modulator inhibits formation of .beta.-AP fibers from monomeric .beta.-AP. This assay can be performed using stirring or shaking to accelerate polymerization, thereby increasing the speed of the assay. Moreover the assay can be adapted to a 96-well plate format to screen multiple compounds.

Detailed Description Text (254):
.beta.-amyloid aggregation in the absence of any modulators results in enhanced turbidity of the natural .beta.-AP solution (i.e., an increase in the apparent absorbance at 405 nm over time). Accordingly, a solution including an effective inhibitory modulator compound exhibits reduced turbidity as compared to the control sample without the modulator compound (i.e., less apparent absorbance at 405 nm over time as compared to the control sample).

Detailed Description Text (256):
The seeded extension assay can be employed to measure the rate of A.beta. fiber formed in a solution of A.beta. monomer following addition of polymeric A.beta. fiber "seed". The ability of test compounds to prevent further deposition of monomeric A.beta. to previously deposited amyloid is determined using a direct indicator of .beta.-sheet formation using fluorescence. In contrast with the nucleation assay, the addition of seed provides immediate nucleation and continued growth of preformed fibrils without the need for continuous mixing, and thus results in the absence of a lag time before polymerization starts. Since this assay uses static polymerization conditions, the activity of positive compounds in the nucleation assay can be confirmed in this second assay under different conditions and with an additional probe of amyloid structure.

Detailed Description Text (257):
In the seeded extension assay, monomeric A.beta..sub.1-40 is incubated in the presence

of a "seed" nucleus (approximately ten mole percent of A.beta. that has been previously allowed to polymerize under controlled static conditions). Samples of the solution are then diluted in thioflavin T (Th-T). The polymer-specific association of Th-T with A.beta. produces a fluorescent complex that allows the measurement of the extent of fibril formation (Levine, H. (1993) Protein Science 2:404-410). In particular, association of Th-T with aggregated .beta.-AP, but not monomeric or loosely associated .beta.-AP, gives rise to a new excitation (ex) maximum at 450 nm and an enhanced emission (em) at 482 nm, compared to the 385 nm (ex) and 445 nm (em) for the free dye. Small aliquots of the polymerization mixture contain sufficient fibril to be mixed with Th-T to allow the monitoring of the reaction mixture by repeated sampling. A linear growth curve is observed in the presence of excess monomer. The formation of thioflavin T responsive .beta.-sheet fibrils parallels the increase in turbidity observed using the nucleation assay.

Detailed Description Text (260):
.beta.-amyloid aggregation results in enhanced emission of Thioflavin-T. Accordingly, samples including an effective inhibitory modulator compound exhibit reduced emission as compared to control samples without the modulator compound.

Detailed Description Text (262):
Effect of Different Amino Acid Subregions of A.beta. Peptide on the Inhibitory Activity of .beta.-Amyloid Modulator Compounds

Detailed Description Text (263):
To determine the effect of various subregions of A.beta..sub.1-40 on the inhibitory activity of a a .beta.-amyloid modulator, overlapping A.beta. peptide 15 mers were constructed. For each 15 mer, four different amino-terminal modifiers were tested: a cholyl group, an iminobiotinyl group, an N-acetyl neuraminyl group (NANA) and a 5-(and 6-)-carboxyfluoresceinyl group (FICO). The modulators were evaluated in the nucleation and seeded extension assays described in Example 6.

Detailed Description Text (265):
These results indicate that certain subregions of A.beta..sub.1-40, when modified with an appropriate modifying group, are effective at inhibiting the aggregation of A.beta..sub.1-40. A cholyl group was an effective modifying group for several subregions. Cholic acid alone was tested for inhibitory activity but had no effect on A.beta. aggregation. The A.beta..sub.6-20 subregion exhibited high levels of inhibitory activity when modified with several different modifying groups (cholyl, NANA, iminobiotinyl), with cholyl-A.beta..sub.6-20 (PPI-264) being the most active form. Accordingly, this modulator compound was chosen for further analysis, described in Example 8.

Detailed Description Text (267):
Identification of a Five Amino Acid Subregion of A.beta. Peptide Sufficient for Inhibitory Activity of a .beta.-Amyloid Modulator Compound

Detailed Description Text (268):
To further delineate a minimal subregion of cholyl-A.beta..sub.6-20 sufficient for inhibitory activity, a series of amino terminal and carboxy terminal amino acid deletions of cholyl-A.beta..sub.6-20 were constructed. The modulators all had the same cholyl amino-terminal modification. Additionally, for the peptide series having carboxy terminal deletions, the carboxy terminus was further modified to an amide. The modulators were evaluated as described in Example 7 and the results are summarized below in Table III, wherein the data is presented as described in Example 7.

Detailed Description Text (269):
These results indicate that activity of the modulator is maintained when amino acid residue 6 is removed from the amino terminal end of the modulator (i.e., cholyl-A.beta..sub.7-20 retained activity) but activity is lost as the peptide is deleted further at the amino-terminal end by removal of amino acid position 7 through to amino acid position 12 (i.e., cholyl-A.beta..sub.8-20 through cholyl-A.beta..sub.13-20 did inhibit the plateau level of A.beta. aggregation). However, further deletion of amino acid position 13 resulted in a compound (i.e., cholyl-A.beta..sub.4-20) in which inhibitory activity is restored. Furthermore, additional deletion of amino acid position 14 (i.e., cholyl-A.beta..sub.15-20) or positions 14 and 15 (i.e., cholyl-A.beta..sub.16-20) still maintained inhibitory activity. Thus, amino terminal deletions of A.beta..sub.6-20 identified A.beta..sub.16-20 as a minimal subregion which is sufficient for inhibitory activity when appropriately modified. In contrast, carboxy terminal deletion of amino acid

position 20 resulted in loss of activity which was not fully restored as the peptide was deleted further at the carboxy-terminal end. Thus, maintenance of position 20 within the modulator may be important for inhibitory activity.

Detailed Description Text (271):
Identification of a Four Amino Acid Subregion of A.beta. Peptide Sufficient for Inhibitory Activity of a .beta.-Amyloid Modulator Compound

Detailed Description Text (272):
In this example, the smallest effective modulator identified in the studies described in Example 8, cholyl-A.beta..sub.16-20 (PPI-350), was analyzed further. Additional amino- and carboxy-terminal deletions were made with cholyl-A.beta..sub.16-20, as well as an amino acid substitution (Val.sub.18 ->Thr), to identify the smallest region sufficient for the inhibitory activity of the modulator. A peptide comprised of five alanine residues, (Ala).sub.5 ; SEQ ID NO:35, modified at its amino-terminus with cholic acid, was used as a specificity control. The modulators were evaluated as described in Example 7 and the results are summarized below in Table IV, wherein the data is presented as described in Example 7.

Detailed Description Text (273):
As shown in Table IV, cholyl-A.beta..sub.16-20 (PPI-350) and cholyl-A.beta..sub.17-21 (PPI-368) both exhibited inhibitory activity, indicating that the four-amino acid minimal subregion of positions 17-20 is sufficient for inhibitory activity. Loss of position 20 (e.g., in PPI-366 and PPI-321) resulted in loss of inhibitory activity, demonstrating the importance of position 20. Moreover, mutation of valine at position 18 to threonine (in PPI-369) also resulted in loss of activity, demonstrating the importance of position 18. In contrast, mutation of phenylalanine at position 19 to alanine (cholyl-A.beta..sub.16-20 Phe.sub.19- >Ala; PPI-370) resulted in a compound which still retained detectable inhibitory activity. Accordingly, the phenylalanine at position 19 is more amenable to substitution, preferably with another hydrophobic amino acid residue. Cholyl-penta-alanine; SEQ ID NO:35 (PPI-365) showed no inhibitory activity, demonstrating the specificity of the A.beta. peptide portion of the modulator. Moreover, unmodified A.beta..sub.16-20 (PPI-377) was not inhibitory, demonstrating the functional importance of the amino-terminal modifying group. The specific functional group influenced the activity of the modulator. For example, iminobiotinyl-A.beta..sub.16-20 (PPI-374) exhibited inhibitory activity similar to cholyl-A.beta..sub.16-20, whereas an N-acetyl neuraminic acid (NANA)-modified A.beta..sub.16-20 was not an effective inhibitory modulator (not listed in Table IV). A C-terminal amide derivative of cholyl-A.beta..sub.16-20 (PPI-319) retained high activity in delaying the lag time of aggregation, indicating that the carboxy-terminus of the modulator can be derivatized without loss of inhibitory activity. Although this amide-derivatized compound did not inhibit the overall plateau level of aggregation over time, the compound was not tested at concentrations higher than mole 33%. Higher concentrations of the amide-derivatized compound are predicted to inhibit the overall plateau level of aggregation, similar to cholyl-A.beta..sub.16-20 (PPI-350).

Detailed Description Text (275):
Effect of .beta.-Amyloid Modulators on the Neurotoxicity of Natural .beta.-Amyloid Peptide Aggregates

Detailed Description Text (276):
The neurotoxicity of natural .beta.-amyloid peptide aggregates, in either the presence or absence of a .beta.-amyloid modulator, is tested in a cell-based assay using either a rat or human neuronally-derived cell line (PC-12 cells or NT-2 cells, respectively) and the viability indicator 3,(4,4-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT). (See e.g., Shearman, M. S. et al. (1994) Proc. Natl. Acad. Sci. USA 91:1470-1474; Hansen, M. B. et al. (1989) J. Immun. Methods 119:203-210 for a description of similar cell-based viability assays). PC-12 is a rat adrenal pheochromocytoma cell line and is available from the American Type Culture Collection, Rockville, Md. (ATCC CRL 1721). MTT (commercially available from Sigma Chemical Co.) is a chromogenic substrate that is converted from yellow to blue in viable cells, which can be detected spectrophotometrically.

Detailed Description Text (277):
To test the neurotoxicity of natural .beta.-amyloid peptides, stock solutions of fresh A.beta. monomers and aged A.beta. aggregates were first prepared. A.beta..sub.1-40 in 100% DMSO was prepared from lyophilized powder and immediately diluted in one half the final volume in H.sub.2 O and then one half the final volume in 2.times. PBS so that a final concentration of 200 .mu.M peptide, 4% DMSO is achieved. Peptide prepared in this

way and tested immediately on cells is referred to as "fresh" A.beta. monomer. To prepare "aged" A.beta. aggregates, peptide solution was placed in a 1.5 ml Eppendorf tube and incubated at 37.degree. C. for eight days to allow fibrils to form. Such "aged" A.beta. peptide can be tested directly on cells or frozen at -80.degree. C. The neurotoxicity of fresh monomers and aged aggregates were tested using PC12 and NT2 cells. PC12 cells were routinely cultured in Dulbeco's modified Eagle's medium (DMEM) containing 10% horse serum, 5% fetal calf serum, 4 mM glutamine, and 1% gentamycin. NT2 cells were routinely cultured in OPTI-MEM medium (GIBCO BRL CAT. #31985) supplemented with 10% fetal calf serum, 2 mM glutamine and 1% gentamycin. Cells were plated at 10-15,000 cells per well in 90 .mu.l of fresh medium in a 96-well tissue culture plate 3-4 hours prior to treatment. The fresh or aged A.beta. peptide solutions (10 .mu.L) were then diluted 1:10 directly into tissue culture medium so that the final concentration was in the range of 1-10 .mu.M peptide. Cells are incubated in the presence of peptide without a change in media for 48 hours at 37.degree. C. For the final three hours of exposure of the cells to the .beta.-AP preparation, MTT was added to the media to a final concentration of 1 mg/ml and incubation was continued at 37.degree. C. Following the two hour incubation with MTT, the media was removed and the cells were lysed in 100 .mu.L isopropanol/0.4N HCl with agitation. An equal volume of PBS was added to each well and the plates were agitated for an additional 10 minutes. Absorbance of each well at 570 nm was measured using a microtiter plate reader to quantitate viable cells.

Detailed Description Text (279):
To determine the effect of a .beta.-amyloid modulator compound on the neurotoxicity of A.beta..sub.1-40 aggregates, a modulator compound, cholyl-A.beta..sub.6-20 (PPI-264), was preincubated with A.beta..sub.1-40 monomers under standard nucleation assay conditions as described in Example 6 and at particular time intervals post-incubation, aliquots of the .beta.-AP/modulator solution were removed and 1) the turbidity of the solution was assessed as a measure of aggregation and 2) the solution was applied to cultured neuronal cells for 48 hours at which time cell viability was assessed using MTT to determine the neurotoxicity of the solution. The results of the turbidity analysis are shown in FIG. 4, panels A, B and C. In panel A, A.beta..sub.1-40 and cholyl-A.beta..sub.6-20 were both present at 64 .mu.M. In panel B, A.beta..sub.1-40 was present at 30 .mu.M and cholyl-A.beta..sub.6-20 was present at 64 .mu.M. In panel C, A.beta..sub.1-40 was present at 10 .mu.M and cholyl-A.beta..sub.6-20 was present at 64 .mu.M. These data show that an equimolar amount of cholyl-A.beta..sub.6-20 is effective at inhibiting aggregation of A.beta..sub.1-40 (see FIG. 4, panel A) and that as the concentration of A.beta..sub.1-40 is reduced, the amount of detectable aggregation of the A.beta..sub.1-40 monomer is correspondingly reduced (compare FIG. 4, panels B and C with panel A). The corresponding results of the neurotoxicity analysis are shown in FIG. 4, panels D, E, and F. These results demonstrate that the .beta.-amyloid modulator compound not only inhibits aggregation of A.beta..sub.1-40 monomers but also inhibits the neurotoxicity of the A.beta..sub.1-40 solution, illustrated by the reduced percent toxicity of the cells when incubated with the A.beta..sub.1-40 /modulator solution as compared to A.beta..sub.1-40 alone (see e.g., FIG. 4, panel D). Moreover, even when A.beta..sub.1-40 aggregation was not detectable as measured by light scattering, the modulator compound inhibited the neurotoxicity of the A.beta..sub.1-40 solution (see FIG. 4, panels E and F). Thus, the formation of neurotoxic A.beta..sub.1-40 aggregates precedes the formation of insoluble aggregates detectable by light scattering and the modulator compound is effective at inhibiting the inhibiting the formation and/or activity of these neurotoxic aggregates. Similar results were seen with other modulator compounds, such as iminobiotinyl-A.beta..sub.6-20 (PPI-267), cholyl-A.beta..sub.16-20 (PPI-350) and cholyl-A.beta..sub.16-20 -amide (PPI-319).

Detailed Description Text (280):
Additionally, the .beta.-amyloid modulator compounds have been demonstrated to reduce the neurotoxicity of preformed A.beta..sub.1-40 aggregates. In these experiments, A.beta..sub.1-40 aggregates were preformed by incubation of the monomers in the absence of any modulators. The modulator compound was then incubated with the preformed A.beta..sub.1-40 aggregates for 24 hours at 37.degree. C., after which time the .beta.-AP/modulator solution was collected and its neurotoxicity evaluated as described above. Incubation of preformed A.beta..sub.1-40 aggregates with the modulator compound prior to applying the solution to neuronal cells resulted in a decrease in the neurotoxicity of the A.beta..sub.1-40 solution. These results suggest that the modulator can either bind to A.beta. fibrils or soluble aggregate and modulate their inherent neurotoxicity or that the modulator can perturb the equilibrium between monomeric and aggregated forms of A.beta..sub.1-40 in favor of the non-neurotoxic form.

Detailed Description Text (282):
Characterization of Additional .beta.-Amyloid Modulator Compounds

Detailed Description Text (283):
In this example, additional modulator compounds designed based upon amino acids 17-20 of A.beta., LVFF; SEQ ID NO:12 (identified in Example 9), were prepared and analyzed to further delineate the structural features necessary for inhibition of .beta.-amyloid aggregation. Types of compounds analyzed included ones having only three amino acid residues of an A.beta. aggregation core domain, compounds in which the amino acid residues of an A.beta. aggregation core domain were rearranged or in which amino acid substitutions had been made, compounds modified with a carboxy-terminal modifying group and compounds in which the modifying group had been derivatized. Abbreviations used in this example are: h- (free amino terminus), -oh (free carboxylic acid terminus), -nh.sub.2 (amide terminus), CA (cholyl, the acyl portion of cholic acid), NANA (N-acetyl neuraminyl), IB (iminobiotinyl), .beta.A (.beta.-alanyl), DA (D-alanyl), Adp (aminoethyldibenzofuranylpropanoic acid), Aic (3-(O-aminoethyl-iso)-cholyl, a derivative of cholic acid), IY (iodotyrosyl), o-methyl (carboxy-terminal methyl ester), N-me (N-methyl peptide bond), DeoxyCA (deoxycholyl) and LithoCA (lithocholyl).

Detailed Description Text (284):
Modulator compounds having an Aic modifying group at either the amino- or carboxy-terminus (e.g., PPI-408 and PPI-418) were synthesized using known methods (see e.g., Wess, G. et al. (1993) Tetrahedron Letters, 34:817-822; Wess, G. et al. (1992) Tetrahedron Letters 33:195-198). Briefly, 3-iso-O-(2-aminoethyl)-cholic acid (3.beta.-(2-aminoethoxy)-7.alpha.,12.alpha.-dihydroxy-5.beta.-cholanoic acid) was converted to the FMOC-protected derivative using FMOC-OSu (the hydroxysuccinimide ester of the FMOC group, which is commercially available) to obtain a reagent that was used to introduce the cholic acid derivative into the compound. For N-terminal introduction of the cholic acid moiety, the FMOC-protected reagent was coupled to the N-terminal amino acid of a solid-phase peptide in the standard manner, followed by standard FMOC-deprotection conditions and subsequent cleavage from the resin, followed by HPLC purification. For C-terminal introduction of the cholic acid moiety, the FMOC-protected reagent was attached to 2-chlorotrityl chloride resin in the standard manner. This amino acyl derivatized resin was then used in the standard manner to synthesize the complete modified peptide.

Detailed Description Text (285):
The modulators were evaluated in the nucleation and seeded extension assays described in Example 6 and the results are summarized below in Table V. The change in lag time (.DELTA.Lag) is presented as the ratio of the lag time observed in the presence of the test compound to the lag time of the control. Data are reported for assays in the presence of 100 mole % inhibitor relative to the concentration of A.beta..sub.1-40, except for PPI-315, PPI-348, PPI-380, PPI-407 and PPI-418, for which the data is reported in the presence of 33 mole % inhibitor. Inhibition (% I.sub.nucl'n) is listed as the percent reduction in the maximum observed turbidity in the control at the end of the assay time period. Inhibition in the extension assay (% I.sub.ext'n) is listed as the percent reduction of thioflavin-T fluorescence of .beta.-structure in the presence of 25 mole % inhibitor. Compounds with a % I.sub.nucl'n of at least 30% are highlighted in bold.

Detailed Description Text (287):
The results shown in Table V demonstrate that at an effective modulator compound can comprise as few as three A.beta. amino acids residues (see PPI-394, comprising the amino acid sequence VFF, which corresponds to A.beta..sub.18-20, and PPI-395, comprising the amino acid sequence FFA, which corresponds to A.beta..sub.19-21). The results also demonstrate that a modulator compound having a modulating group at its carboxy-terminus is effective at inhibiting A.beta. aggregation (see PPI-408, modified at its C-terminus with Aic). Still further, the results demonstrate that the cholyl group, as a modulating group, can be manipulated while maintaining the inhibitory activity of the compounds (see PPI-408 and PPI-418, both of which comprise the cholyl derivative Aic). The free amino group of the Aic derivative of cholic acid represents a position at which a chelation group for .sup.99m Tc can be introduced, e.g., to create a diagnostic agent. Additionally, the ability to substitute iodotyrosyl for phenylalanine at position 19 or 20 of the A.beta. sequence (see PPI-396 and PPI-397) while maintaining the ability of the compound to inhibit A.beta. aggregation indicates that the compound could be labeled with radioactive iodine, e.g., to create a diagnostic agent, without loss of the inhibitory activity of the compound.

Detailed Description Text (288):

Finally, compounds with inhibitory activity were created using A.beta. derived amino acids but wherein the amino acid sequence was rearranged or had a substitution with a non-A.beta.-derived amino acid. Examples of such compounds include PPI-426, in which the sequence of A.beta..sub.17-21 (LVFFA SEQ ID NO:11) has been rearranged (FFVLA SEQ ID NO:21), PPI-372, in which the sequence of A.beta..sub.16-20 (KLVFF SEQ ID NO:10) has been rearranged (FKFVL SEQ ID NO:29), and PPI-388, -389 and -390, in which the sequence of A.beta..sub.17-21 (LVFFA SEQ ID NO:11) has been substituted at position 17, 18 or 19, respectively, with an alanine residue (AVFFA (SEQ ID NO:25) for PPI-388, LAFFA (SEQ ID NO:13) for PPI-389 and LVAFA (SEQ ID NO:33) for PPI-390). The inhibitory activity of these compounds indicate that the presence in the compound of an amino acid sequence directly corresponding to a portion of A.beta. is not essential for inhibitory activity, but rather suggests that maintenance of the hydrophobic nature of this core region, by inclusion of amino acid residues such as phenylalanine, valine, leucine, regardless of their precise order, can be sufficient for inhibition of A.beta. aggregation.

Detailed Description Text (290):
Characterization of .beta.-Amyloid Modulator Compounds Comprising an Unmodified .beta.-Amyloid Peptide

Detailed Description Text (291):
To examine the ability of unmodified A.beta. peptides to modulate aggregation of natural .beta.-AP, a series of A.beta. peptides having amino- and/or carboxy terminal deletions as compared to A.beta..sub.1-40, or having internal amino acids deleted (i.e., noncontiguous peptides), were prepared. One peptide (PPI-220) had additional, non-A.beta.-derived amino acid residues at its amino-terminus. These peptides all had a free amino group at the amino-terminus and a free carboxylic acid at the carboxy-terminus. These unmodified peptides were evaluated in assays as described in Example 7. The results are summarized below in Table VI, wherein the data is presented as described in Example 7. Compounds exhibiting at least a 1.5 fold increase in lag time are highlighted in bold.

Detailed Description Text (292):
The results shown in Table VI demonstrate that limited portions of the A.beta. sequence can have a significant inhibitory effect on natural .beta.-AP aggregation even when the peptide is not modified by a modifying group. Preferred unmodified peptides are A.beta..sub.6-20 (PPI-226), A.beta..sub.16-30 (PPI-228), A.beta..sub.1-20, 26-40 (PPI-249) and EEVVHHHHQQ-A.beta..sub.16-20 (PPI-220), the amino acid sequences of which are shown in SEQ ID NOs:4, 14, 15, and 16, respectively.

Detailed Description Paragraph Table (1):
TABLE I _____ Can- Effect in Effect in didate shaken Seeded In- A.beta. Amino Modifying plate Static hibitor Acids Reagent assay Assay* _____ 174 A.beta.1-15 Cholic acid Complete ++ inhibition at 100% conc 176 A.beta.1-15 Diethylene- Decreased ++ triamine penta Plateau acetic acid 180 A.beta.1-15 (-)-Menthoxy None ++ acetic acid 190 A.beta.1-15 Fluorescein Decreased ++ carboxylic acid Plateau (FICO) 220 A.beta.16-40 NH.sub.2 --EVHHHHQQK- Complete ++ mutant [A.beta.(16-40)]-COOH inhibi- tion at (SEQ ID NO:16) 100%, increased lag at 10% 224 A.beta.1-40 F.sub.19 F.sub.20 -> T.sub.19 T.sub.20 Increased ++ mutant lag 233 A6.beta.-20 Acetic acid acceler- ++ ated aggrega- tion at 10% conc _____ *++ = A strong inhibitor of aggregation. The rate of aggregation in the presence of the inhibitor was decreased compared to the control by at least 30.50%

Detailed Description Paragraph Table (5):
TABLE V _____ N-Term. C-Term. % Ref. # Mod. Peptide Mod. .DELTA.Lag % I.sub.nucl'n I.sub.ext'n _____ PPI-293 CA -- oh 1.0 0 ND* PPI-315 CA HQKLVFF nh.sub.2 1.1 5** ND PPI-316 NANA HQKLVFF nh.sub.2 1.5 -15 ND PPI-319 CA KLVFF nh.sub.2 5.4 70 52 PPI-339 CA HDSGY nh.sub.2 1.1 -18 ND PPI-348 CA HQKLVFF oh 2.0 70** ND PPI-349 CA QKLVFF oh >5 100 56 PPI-350 CA KLVFF oh 1.8 72 11 PPI-365 CA AAAAA oh 0.8 -7 0 PPI-366 CA QKLVF oh 3.1 -23 ND PPI-368 CA LVFFA oh >5 100 91 PPI-369 CA KLTFF oh 1.1 -16 44 PPI-370 CA KLVAF oh 2.6 73 31 PPI-371 CA KLVFF(.beta.A) oh 2.5 76 80 PPI-372 CA FKFVL oh 0.8 45 37 PPI-373 NANA KLVFF oh 0.9 16 8 PPI-374 IB KLVFF oh 1.3 86 0 PPI-375 CA KTVFF oh 1.2 18 21 PPI-377 h- KLVFF oh 1.1 0 8 PPI-379 CA LVFFAE oh 1.4 55 16 PPI-380 CA LVFF oh 1.8 72** 51 PPI-381 CA LVFF(DA) oh 2.3 56 11 PPI-382 CA LVFFA nh.sub.2 1.0 -200 91 PPI-383 h-DDIIL- VFF oh 0.4 14 0 (Adp) PPI-386 h- LVFFA oh 1.0 15 11 PPI-387 h- KLVFF nh.sub.2 1.3 -9 39 PPI-388 CA AVFFA oh 1.4 68 44 PPI-389 CA LAFFA oh 1.5 47 66 PPI-390 CA LVAFA oh 2.7 25 0 PPI-392 CA VFFA oh 2.0 76 10 PPI-393 CA LVF oh 1.3 1 0 PPI-394 CA VFF oh 1.8 55 0 PPI-395 CA

FFA oh 1.0 51 6 PPI-396 CA LV(IY)FA oh >5 100 71 PPI-401 CA LVFFA o-methyl ND ND 0 PPI-405 h- LVFFA nh.sub.2 1.3 11 70 PPI-407 CA LVFFK oh >5 100** 85 PPI-408 h- LVFFA (Aic)-oh 3.5 46 3 PPI-418 h-(Aic) LVFFA oh >5 100** 87 PPI-426 CA FFVLA oh >5 100 89 PPI-391 CA LVFAA oh 1.6 40 ND PPI-397 CA LVF(IY)A oh >5 95 ND PPI-400 CA AVAFA oh 1.0 -15 ND PPI-403 *** HQKLVFF oh 1.4 -75 0 PPI-404 **** LKLVFF oh 1.8 -29 7 PPI-424 DeoxyCA LVFFA oh 3.0 -114 82 PPI-425 LithoCA LVFFA oh 2.8 -229 0 PPI-428 CA FF oh 1.7 -78 15 PPI-429 CA FFV oh 2.2 -33 7 PPI-430 CA FFVL oh 4.1 33 75 PPI-433 CA LVFFA oh 2.8 27 ND (all D amino acids) PPI-435 t-Boc LVFFA oh 3.0 -5 ND PPI-438 CA GFF oh 1.0 0 ND

*ND = not done **33 mol %

***hDDIII(N-Me-Val)DLL(Adp) ****hDDII(N-Me-Leu)VEH(Adp)

Detailed Description Paragraph Table (7):

SEQ ID NO: Amino Acids Peptide Sequence

1 43 amino acids A.beta..sub.1-43 2 103 amino acids APP C-terminus 3 43 amino acids A.beta..sub.1-43 (19, 20 mutated) 4 HDSGYEVHHQKLVFF A.beta..sub.6-20 5 HQKLVFFA A.beta..sub.14-21 6 HQKLVFF A.beta..sub.14-20 7 QKLVFFA A.beta..sub.15-21 8 QKLVFF A.beta..sub.15-20 9 KLVFFA A.beta..sub.16-21 10 KLVFF A.beta.16-20 11 LVFFA A.beta..sub.17-21 12 LVFF A.beta..sub.17-20 13 LAFFA A.beta..sub.17-21 (V.sub.18 .fwdarw.A) 14 KLVFFAEDVGSNKGA A.beta..sub.16-30 15 35 amino acids A.beta..sub.1-20, 26-40 16 35 amino acids EEVVHHHHQQ-.beta.AP.sub.16-40 17 AGAAAAGA PrP peptide 18 AILSS amylin peptide 19 VFF A.beta..sub.18-20 20 FFA A.beta..sub.19-21 21 FFVLA A.beta..sub.17-21 (scrambled) 22 LVFFK A.beta..sub.17-21 (A.sub.21 .fwdarw.K) 23 LV(IY)FA A.beta..sub.17-21 (F.sub.19 .fwdarw.IY) 24 VFFA A.beta..sub.18-21 25 AVFFA A.beta..sub.17-21 (L.sub.17 .fwdarw.A) 26 LVF(IY)A A.beta..sub.17-21 (F.sub.20 .fwdarw.IY) 27 LVFFAE A.beta..sub.17-22 28 FFVL A.beta..sub.17-20 (scrambled) 29 FKFVL A.beta..sub.16-20 (scrambled) 30 KLVAF A.beta..sub.16-20 (F.sub.19 .fwdarw.A) 31 KLVFF(.beta.A) A.beta..sub.16-21 (A.sub.21 .fwdarw..beta.A) 32 LVFF(DA) A.beta..sub.17-21 (A.sub.21 .fwdarw.DA)

Detailed Description Paragraph Table (8):

SEQUENCE LISTING (1) GENERAL INFORMATION: (iii) NUMBER OF SEQUENCES: 40 (2) INFORMATION FOR SEQ ID NO:1: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 43 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1: AspAlaGluPheArgHisAspSerGlyTyrGluValHisHisGlnLys 151015 LeuValPhePheAlaGluAspValGlySerAsnLysGlyAlaIleIle 202530 GlyLeuMetValGlyGlyValValIleAlaThr 3540 (2) INFORMATION FOR SEQ ID NO:2: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 103 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2: GluValLysMetAspAlaGluPheArgHisAspSerGlyTyrGluVal 151015 HisHisGlnLysLeuValPhePheAlaGluAspValGlySerAsnLys 202530 GlyAlaIleIleGlyLeuMetValGlyGlyValValIleAlaThrVal 354045 IleValIleThrLeuValMetLeuLysLysLysGlnTyrThrSerIle 505560 HisHisGlyValValGluValAspAlaAlaValThrProGluGluArg 65707580 HisLeuSerLysMetGlnGlnAsnGlyTyrGluAsnProThrTyrLys 859095 PhePheGluGlnMetGlnAsn 100 (2) INFORMATION FOR SEQ ID NO:3: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 43 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (ix) FEATURE: (A) NAME/KEY: Modified site (B) LOCATION: 19 (D) OTHER INFORMATION: /note= Xaa is a hydrophobic amino acid (ix) FEATURE: (A) NAME/KEY: Modified site (B) LOCATION: 20 (D) OTHER INFORMATION: /note= Xaa is a hydrophobic amino acid (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3: AspAlaGluPheArgHisAspSerGlyTyrGluValHisHisGlnLys 151015 LeuValXaaXaaAlaGluAspValGlySerAsnLysGlyAlaIleIle 202530 GlyLeuMetValGlyGlyValValIleAlaThr 3540 (2) INFORMATION FOR SEQ ID NO:4: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 15 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4: HisAspSerGlyTyrGluValHisHisGlnLysLeuValPhePhe 51015 (2) INFORMATION FOR SEQ ID NO:5: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 8 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5: HisGlnLysLeuValPhePheAla (2) INFORMATION FOR SEQ ID NO:6: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 7 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6: HisGlnLysLeuValPhePhe 5 (2) INFORMATION FOR SEQ ID NO:7: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 7 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7: GlnLysLeuValPhePheAla 5 (2) INFORMATION FOR SEQ ID NO:8: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 6 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8: GlnLysLeuValPhePhe 5 (2) INFORMATION FOR SEQ ID NO:9: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 6 amino

acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9: LysLeuValPhePheAla 5 (2) INFORMATION FOR SEQ ID NO:10: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10: LysLeuValPhePhe 5 (2) INFORMATION FOR SEQ ID NO:11: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11: LeuValPhePheAla 5 (2) INFORMATION FOR SEQ ID NO:12: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 4 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12: LeuValPhePhe (2) INFORMATION FOR SEQ ID NO:13: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13: LeuAlaPhePheAla 15 (2) INFORMATION FOR SEQ ID NO:14: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 15 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14: LysLeuValPhePheAlaGluAspValGlySerAsnLysGlyAla 151015 (2) INFORMATION FOR SEQ ID NO:15: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 35 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15: AspAlaGluPheArgHisAspSerGlyTyrGluValHisHisGlnLys 151015 LeuValPhePheSerAsnLysGlyAlaIleIleGlyLeuMetValGly 202530 GlyValVal 35 (2) INFORMATION FOR SEQ ID NO:16: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 35 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16: GluGluValValHisHisHisHisGlnGlnLysLeuValPhePheAla 151015 GluAspValGlySerAsnLysGlyAlaIleIleGlyLeuMetValGly 202530 GlyValVal 35 (2) INFORMATION FOR SEQ ID NO:17: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 8 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17: AlaGlyAlaAlaAlaAlaGlyAla 15 (2) INFORMATION FOR SEQ ID NO:18: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18: AlaIleLeuSerSer 15 (2) INFORMATION FOR SEQ ID NO:19: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 3 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19: ValPhePhe 1 (2) INFORMATION FOR SEQ ID NO:20: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 3 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20: PhePheAla 1 (2) INFORMATION FOR SEQ ID NO:21: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21: PhePheValLeuAla 15 (2) INFORMATION FOR SEQ ID NO:22: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22: LeuValPhePheLys 15 (2) INFORMATION FOR SEQ ID NO:23: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid

Detailed Description Paragraph Table (9):
(D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (ix) FEATURE: (A) NAME/KEY: Modified site (B) LOCATION: 3 (D) OTHER INFORMATION: /note= Xaa is iodotyrosyl (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23: LeuValXaaPheAla 15 (2) INFORMATION FOR SEQ ID NO:24: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 4 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24: ValPhePheAla 1 (2) INFORMATION FOR SEQ ID NO:25: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25: AlaValPhePheAla 15 (2) INFORMATION FOR SEQ ID NO:26: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (ix) FEATURE: (A) NAME/KEY: Modified site (B) LOCATION: 4 (D) OTHER INFORMATION: /note= Xaa is iodotyrosyl (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26: LeuValPheXaaAla 1 (2) INFORMATION FOR SEQ ID NO:27: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 6 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27: LeuValPhePheAlaGlu 15 (2) INFORMATION FOR SEQ ID NO:28: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 4 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28: PhePheValLeu 15 (2) INFORMATION FOR SEQ ID NO:29: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29: PheLysPheValLeu 15 (2) INFORMATION FOR SEQ ID NO:30: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30: LysLeuValAlaPhe 15 (2) INFORMATION FOR SEQ ID NO:31: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 6 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (ix)

FEATURE: (A) NAME/KEY: Modified site (B) LOCATION: 6 (D) OTHER INFORMATION: /note= Xaa is beta-alanyl (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31: LysLeuValPhePheXaa 1 (2) INFORMATION FOR SEQ ID NO:32: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (ix) FEATURE: (A) NAME/KEY: Modified site (B) LOCATION: 5 (D) OTHER INFORMATION: /note= Xaa is D-alanyl (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32: LeuValPhePheXaa 1 (2) INFORMATION FOR SEQ ID NO:33: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33: LeuValAlaPheAla 15 (2) INFORMATION FOR SEQ ID NO:34: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (ix) FEATURE: (A) NAME/KEY: Modified-site (B) LOCATION: 5 (D) OTHER INFORMATION: /note=aminoethyldibenzofuranyl- proprionic acid modification (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34: AspAspIleIleLeu 15 (2) INFORMATION FOR SEQ ID NO:35: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35: AlaAlaAlaAlaAla 15 (2) INFORMATION FOR SEQ ID NO:36: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 35 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36: HisAspSerGlyTyrGluValHisHisGlnLysLeuValPhePheAla 151015 GluAspValGlySerAsnLysGlyAlaIleIleGlyLeuMetValGly 202530 GlyValVal 35 (2) INFORMATION FOR SEQ ID NO:37: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 15 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37: GluValHisHisGlnLysLeuValPhePheAlaGluAspValGly 151015 (2) INFORMATION FOR SEQ ID NO:38: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 35 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38: AspAlaGluPheArgHisAspSerGlyTyrGluValHisHisGlnLys 151015 LeuValPhePheAlaGluAspValGlyIleIleGlyLeuMetValGly 202530 GlyValVal 35 (2) INFORMATION FOR SEQ ID NO:39: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 35 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39: AspAlaGluPheArgHisAspSerGlyTyrGluValHisHisGlnAla 151015 GluAspValGlySerAsnLysGlyAlaIleIleGlyLeuMetValGly 202530 GlyValVal 35 (2) INFORMATION FOR SEQ ID NO:40: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 35 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40: AspAlaGluPheArgGluValHisHisGlnLysLeuValPhePheAla 151015 GluAspValGlySerAsnLysGlyAlaIleIleGlyLeuMetValGly 202530 GlyValVal 35

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Barrow, Colin J. and Michael G. Zagorski (1991) "Solution Structures of .beta. Peptide and Its Constituent Fragments: Relation to Amyloid Deposition" Science 253: 179-182.

Other Reference Publication (2):
Barrow, Colin J. et al. (1992) "Solution Conformations and Aggregational Properties of Synthetic Amyloid .beta.-Peptides of Alzheimer's Disease: Analysis of Circular Dichroism Spectra" J. Mol. Biol. 225: 1075-1093.

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Come, Jon H. et al. (1993) "A Kinetic Model for Amyloid Formation in the Prion Diseases: Importance of Seeding" Proc. Natl. Acad. Sci. USA 90: 5959-5963.

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Fabian, Heinz et al. (1993) "Comparative Analysis of Human and Dutch-Type Alzheimer .beta.-Amyloid Peptides by Infrared Spectroscopy and Circular Dichroism" Biochemical and Biophysical Research Communications 191(1): 232-239.

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Flood, J.F. et al., (1994) "Topography of a Binding Site for Small Amnestic Peptides Deduced from Structure-Activity Studies: Relation to Amnestic Effect of Amyloid .beta. Protein," Proc. Natl. Acad. Sci. USA vol. 91, pp. 380-384.

Other Reference Publication (13):
Fraser, Paul E. et al. (1992) "Fibril Formation by Primate, Rodent, and Dutch-Hemorrhagic Analogues of Alzheimer Amyloid .beta.-Protein" Biochemistry 31: 10716-10723.

Other Reference Publication (14):
Gorevic, PD et al. (1987) "Ten to Fourteen Residue Peptides of Alzheimer's Disease Protein are Sufficient for Amyloid Fibril Formation and Its Characteristic Xray Diffraction Pattern" Biochemical and Biophysical Research Communications 147(2): 854-862.

Other Reference Publication (15):
Gowing, Eric et al. (1994) "Chemical Characterization of A.beta. 17-42 Peptide, a Component of Diffuse Amyloid Deposits of Alzheimer Disease" J. Biol. Chem. 269(15): 10987-10990.

Other Reference Publication (16):
Halverson, Kurt et al. (1990) "Molecular Determinants of Amyloid Deposition in Alzheimer's Disease: Conformational Studies of Synthetic .beta.-Protein Fragments" Biochemistry 29(11): 2639-2644.

Other Reference Publication (17):
Hansen, Morten B. et al. (1989) "Re-examination and Further Development of a Precise and Rapid Dye Method for Measuring Cell Growth/Cell Kill" J. Immunol. Meth. 119: 203-210.

Other Reference Publication (18):
Hardy, John A. and Gerald A. Higgins (1992) "Alzheimer's Disease: The Amyloid Cascade Hypothesis" Science 256: 184-185.

Other Reference Publication (19):
Hilbich, Caroline et al. (1991) "Aggregation and Secondary Structure of Synthetic Amyloid .beta.A4 Paptides of Alzheimer's Disease" J. Mol. Biol. 218: 149-163.

Other Reference Publication (20):
Hilbich, Caroline et al. (1991) "Human and Rodent Sequence Analogs of Alzheimer's Amyloid .beta.A4 A4 Share Similar Properties and can Be Solubilized in Buffers of pH 7.4" Eur. J. Biochem. 201: 61-69.

Other Reference Publication (21):
Hilibich, Caroline et al. (1992) "Substitutions of Hydrophobic Amino Acids Reduce the Amyloidogenicity of Alzheimer's Disease .beta.A4 Peptides" J. Mol. Biol. 228: 460-473.

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Inouye, H. et al. (1993) "Structure of Beta-Crystallite Assemblies Formed by Alzheimer .beta.-Amyloid Protein Analogs: Analysis by X-ray Diffraction," Chemical Abstracts vol. 119, p. 349, Abstract No. 119.

Other Reference Publication (24):
Jarrett, Joseph T. et al. (1993) "The Carboxy Terminus of the .beta. Amyloid Protein Is Critical for the Seeding of Amyloid Formation: Implications for the Pathogenesis of Alzheimer's Disease" Biochemistry 32(18): 4693-4697.

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Jarrett, Joseph T. et al. (1994) "Models of the .beta. Protein C-Terminus: Differences in Amyloid Structure May Lead to Segregation of `Long` and `Short` Fibrils" Journal of the American chemical Society 116(21): 9741-9742.

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Kelly, Jeffrey W. and Peter T. Lansbury, Jr. (1994) "A Chemical Approach to Elucidate the Mechanism of Transthyretin and .beta.-Protein Amyloid Fibril Formation" Int. J. Exp. Clin. Invest 1: 186-205.

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Kirschner, Daniel A. et al. (1987) "Synthetic Peptide Homologous to .beta. Protein from Alzheimer Disease forms Amyloid-like Fibrils in vitro" Proc. Natl. Acad. Sci. USA 84: 6953-6957.

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Klunk, William E. and Jay W. Pettegrew (1990) "Alzheimer's .beta.-Amyloid Protein Is Covalently Modified When Dissolved in Formic Acid" Journal of Neurochemistry 54(6): 2050-2054.

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Lansbury, Jr., Peter T. (1992) "In Pursuit of the Molecular Strucutre of Amyloid Plaque: New Technology Provides Unexpected and Critical Information" Biochemistry 31(30): 6866-6870.

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Miller, Brian T. et al. (1994) "Identification and Characterization of O-Biotinylated Hydroxy Amino Acid Residues in Peptides" Analytical Biochemistry 219: 240-248.

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Orlando, Ron et al. (1992) "Covalent Modification of Alzheimer's Amyloid .beta.-Peptide in Formic Acid Solutions" Biochemical and Biophysical Research Communications 184(2): 686-691.

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Pike, Christian J. et al. (1993) "Neurodegeneration induced by .beta.-Amyloid Peptides in vitro: The Role of Peptide Assembly State" Journal of Neuroscience 13(4): 1676-1687.

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Pike, Christian J. et al. (1995) "Structure-Activity Analyses of .beta.-Amyloid Peptides: Contributions of the .beta.25-35 Region to Aggregation and Neurotoxity" Journal of Neurochemistry 64(1): 253-265.

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Schwarzman, Alexander L. et al. (1994) "Transthyretin Sequesters Amyloid .beta. Protein and Prevents Amyloid Formation" Proc. Natl. Acad. Sci. USA 91: 8368-8372.

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Shearman, Mark S. et al. (1994) "Inhibition of PC12 Cell Redox Activity is a Specific, Early Indicator of the Mechanism of .beta.-Amyloid-Mediated Cell Death" Proc. Natl. Acad. Sci. USA 91: 1470-1474.

Other Reference Publication (38):
Shen, Chih-Lung et al. (1994) "Effect of Acid Predissolution on Fibril Size and Fibril Flexibility of Synthetic .beta.-Amyloid Peptide" Biophysical Journal 67: 1238-1246.

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Shen, Chih-Lung et al. (1993) "Light Scattering Analysis of Fibril Growth from the Amino-Terminal Fragment .beta.(1-28) of .beta.-Amyloid Peptide" Biophysical Journal 65: 2383-2395.

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Snyder, Seth W. et al. (1994) "Amyloid-.beta. Aggregation: Selective Inhibition of Aggregation in Mixtures of Amyloid with Different Chain Lengths" Biophysical Journal

67: 1216-1228.

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Sonnenberg-Reines, J. et al. (1993) "Biotinylated and Cysteine Modified Peptides as Useful Reagents for Studying the Inhibition of Putative N-terminal B-Amyloid Peptide Enzymes," Society for Neuroscience Abstracts vol. 19 (1-3), p. 861.

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Soreghan, Brian et al. (1994) "Surfactant Properties of Alzheimer's A.beta. Peptides and the Mechanism of Amyloid Aggregation" The Journal of Biological Chemistry 158(46): 28551-28554.

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Sorimachi, Kay and David J. Craik (1994) "Structure Determination of Extracellular Fragments of Amyloid Proteins Involved in Alzheimer's Disease and Dutch-type Hereditary Cerebral Haemorrhage with Amyloidosis" Eur. J. Biochem 219: 237-251.

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Strittmatter, Warren J. et al. (1993) "Binding of Human Apolipoprotein E to Synthetic Amyloid .beta. Peptide: Isoform-Specific Effects and Implications for Late-Onset Alzheimer Disease" Proc. Natl. Acad. Sci. USA 90: 8098-8102.

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Tomiyama, Takami et al. (1994) "Racemization of Asp.sup.23 Residue Affects the Aggregation Properties of Alzheimer Amyloid .beta. Protein Analogues" J. Biol. Chem. 269(14): 10205-10208.

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Tomski, Sharon J. and Regina M. Murphy (1992) "Kinetics of Aggregation of Synthetic .beta.-Amyloid Peptide" Archives of Biochemistry and Biophysics 294(2): 630-638.

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Vyas, S. B. et al. "Characterization of Aggregation in Alzheimer .beta.-protein Using Synthetic Peptide Fragments on Reverse-Phase Matrix," in Peptides, Chemistry and Biology (J.A. Smith and J.E. Rivier, eds.), ESCOM, Leiden, 1992, pp. 278-279.

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Weinreb, Paul H. et al. (1994) "Peptide Models of a Hydrophobic Cluster at the C-Terminus of the .beta.-Amyloid" Journal of the American Chemical Society 116(23): 10835-10836.

CLAIMS:

1. A .beta.-amyloid peptide compound having a structure: .beta.AP.sub.6-20 (SEQ ID NO:4).

2. A .beta.-amyloid peptide compound having a structure: .beta.AP.sub.16-30 (SEQ ID NO:14).

3. A .beta.-amyloid peptide compound having a structure: .beta.AP.sub.1-20, 26-40 (SEQ ID NO:15).

4. A .beta.-amyloid peptide compound having a structure: EEVVHHHHQQ-.beta.AP.sub.16-40 (SEQ ID NO:16).

5. A .beta.-amyloid peptide compound having a structure: A.beta..sub.6-40 (SEQ ID NO:36).

6. A .beta.-amyloid peptide compound having a structure: A.beta..sub.11-25 (SEQ ID NO:37).

7. A .beta.-amyloid peptide compound having a structure: A.beta..sub.1-25, 31-40 (.DELTA.26-30) (SEQ ID NO:38).

8. A .beta.-amyloid peptide compound having a structure: A.beta..sub.1-15, 21-40 (.DELTA.16-20) (SEQ ID NO:39).

9. A .beta.-amyloid peptide compound having a structure: A.beta..sub.1-5, 11-40 (.DELTA.6-10) (SEQ ID NO:40).